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Epstein–Barr virus (*Herpesviridae: Gammaherpesvirinae: Lymphocryptovirus: Human gammaherpesvirus 4*) in Kalmyks and Slavs living in Russia: virus types, *LMP1* oncogene variants, and malignancies

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Introduction. The discovery of the Epstein-Barr virus types (*Herpesviridae: Gammaherpesvirinae: Lymphocryptovirus: Human gammaherpesvirus* 4) (EBV) – EBV-1 and EBV-2, which have different transforming abilities *in vitro*, stimulated the study of their prevalence in populations in order to elucidate the relationship with malignant neoplasms.
The aims of the work are to study the prevalence of EBV-1 and EBV-2 among representatives of 2 ethnic groups of Russia, Kalmyks and Slavs, sequencing analysis of the LMP1 oncogene in virus isolates, and analysis of the correlation between virus types and the incidence of certain forms of tumors.
Materials and methods. DNA samples were isolated from the biological material of oral swabs obtained from ethnic Kalmyks of the Republic of Kalmykia (RK) (n = 50) and Slavs, residents of the Moscow Region (MR) (n = 40). DNA samples were used to amplify EBV DNA, followed by determination of its concentration per 1 cell of washout, amplification of the *LMP1* oncogene in viral samples, their sequencing, and determination of LMP1 protein variants.
Results. It has been established that with the same burden of EBV among representatives of both ethnic groups in the Kalmyk group, the ratio of persons infected with transforming and non-transforming types of the virus was almost the same (EBV-1 – 51%; and EBV-2 – 49%). Meanwhile, in the group of Slavs the transforming EBV-1 type virus dominated (80.6%). The predominance of EBV-1 type in representatives of the Slavs correlated with increased incidence of certain forms of tumors in the population of the MR when compared with similar values in the population of the RK, where both types of the virus were prevalent. Differences between the compared rates of cancer incidence were not statistically significant. Analysis of viral isolates showed a similar set of LMP1 variants in both ethnic groups.
Conclusion. In order to establish the influence of EBV types on the incidence of malignant tumors, additional studies involving representatives of various ethnic groups from different geographical regions are needed.

Keywords: *Epstein–Barr virus (EBV); EBV-1 and EBV-2 types; latent membrane protein 1 (LMP1); sequence analysis; Kalmyks; Slavs; real-time polymerase chain reaction (PT-PCR); tumors with EBV-associated cases*

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НАУЧНАЯ СТАТЬЯ
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Вирус Эпштейна–Барр (*Herpesviridae: Gammaherpesvirinae: Lymphocryptovirus: Human gammaherpesvirus 4*) у калмыков и славян, проживающих на территории России: типы вируса, варианты онкогена *LMP1* и злокачественные опухоли

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Введение. Открытие типов вируса Эпштейна–Барр (*Herpesviridae: Gammaherpesvirinae: Lymphocryptovirus: Human gammaherpesvirus 4*) (ВЭБ) – ВЭБ-1 и ВЭБ-2, обладающих различной трансформирующей способностью *in vitro,* стимулировало изучение их распространённости в популяциях с целью выяснения связи со злокачественными новообразованиями.
Цели работы – изучение распространённости ВЭБ-1 и ВЭБ-2 у представителей 2 этносов России, калмыков и славян, сиквенсный анализ онкогена *LMP1* в изолятах вируса и анализ корреляции между типами вируса и заболеваемостью определенными формами опухолей.
Материалы и методы. Из биологического материала смывов полости рта, полученных от этнических калмыков Республики Калмыкия (РК) (*n* = 50) и славян, жителей Московской области (МО) (*n* = 40), выделяли образцы ДНК. Последние использовали для амплификации ДНК ВЭБ, с последующим определением её концентрации на 1 клетку смыва, амплификацией в вирусных образцах онкогена *LMP1,* их секвенированием и определением белковых вариантов LMP1.
Результаты. Установлено, что при одинаковой нагрузке ВЭБ среди представителей обоих этносов в группе калмыков соотношение лиц, инфицированных трансформирующим и нетрансформирующим типами вируса, было практически одинаковым (ВЭБ-1 – 51%; и ВЭБ-2 – 49%), а в группе славян доминировал трансформирующий тип ВЭБ-1 (80,6%). Доминантное инфицирование представителей славян 1-м типом вируса (ВЭБ-1) коррелировало с повышенными показателями заболеваемости некоторыми формами опухолей у населения МО при сравнении с аналогичными показателями у населения РК, представители которой инфицированы обоими типами вируса. Различия между сравниваемыми показателями онкозаболеваемости не были статистически значимыми. Анализ вирусных изолятов показал близкий набор вариантов LMP1 у обеих этнических групп.
Заключение. С целью установления влияния типов ВЭБ на заболеваемость злокачественными новообразованиями необходимы дополнительные исследования с участием представителей различных этнических групп из разных географических регионов.

Ключевые слова: *вирус Эпштейна–Барр (ВЭБ); калмыки; славяне; типы ВЭБ-1 и ВЭБ-2; LMP1; полимеразная цепная реакция в реальном времени (ПЦР-РВ); сиквенсный анализ; опухоли с встречающимися ВЭБ-ассоциированными случаями*

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Introduction

The Epstein–Barr virus (EBV) (*Herpesviridae: Gammaherpesvirinae: Lymphocryptovirus: Human gammaherpesvirus 4*) is one of the most common human viruses. Formerly, the pathogen was known as *Human herpesvirus 4* (*HHV4*) [1]. The complex EBV genome is composed of a linear double-stranded DNA 170–185 kbp in length and including around 85 possible coding regions [2]. Humans serve as the only natural host for the virus. More than 90% of the adult population of the planet are EBV carriers [3]. Infection occurs in early childhood and is asymptomatic; infected individuals carry the virus for life, usually, without any clinical symptoms. EBV has been implicated in development of a variety of benign or malignant conditions, accounting for 200,000 new cancer cases annually [3, 4]. Unlike other human oncogenic viruses such as human papilloma viruses (*Papillomaviridae: Alphapapillomavirus: Human papillomavirus*), Merkel cell polyomavirus (*Polyomaviridae: Alphapolyomavirus: Human polyomavirus 5*) (MCPyV, MCV), human T-cell lymphotropic virus type 1 (*Retroviridae: Deltaretrovirus. Primate T-lymphotropic virus 1: Human T-lymphotropic virus 1)* (HTLV-1), hepatitis B virus (*Hepadnaviridae: Orthohepadnavirus: Hepatitis B virus*) (HBV), which display their oncogenic potential only toward specific target cells, EBV has a broad cell tropism. It is associated with a wide range of lymphoid and epithelial malignancies. This pathogen is known as a causative agent of 2 diseases – B-cell/T/NK-cell lymphoproliferative disorders (B-LPD and T/NK-LPD). EBV has been found to contribute to development of at least 9 pathogenetically distinct neoplasms. They include Burkitt lymphoma (BL) and Hodgkin lymphoma (HL), diffuse large B-cell lymphoma (DLBCL), plasmablastic lymphoma (PBL), natural killer/T-cell lymphoma (NK/T-cell lymphoma), nasopharyngeal carcinoma (NPC) and gastric carcinoma (GC), leiomyosarcoma and primary effusion lymphoma (PEL), which is always associated with Kaposi's sarcoma-associated herpesvirus (*Herpesviridae: Gammaherpesvirinae: Rhadinovirus: Human gammaherpesvirus 8*) (KSHV) [3]. Out of these nine, five malignancies, including PEL, are B-cell derived. Other malignancies originate from infected T and/or NK cells or from epithelium of the nasal cavity, stomach, and smooth muscles in individuals with immunodeficiency syndrome.

During the evolutionary process, EBV has acquired a unique set of latent, the so-called growth-transforming genes. In different infected substrates, these genes have different patterns of expression referred to as latency types (growth programs) [5]. Viral-genome containing lymphoblastoid cell lines (LCL) demonstrate type III latency including expression of virus-encoded nuclear antigens EBNA-1 (essential, among other things, for EBV episome maintenance), EBNA-2, -3A, -3B, -3C, and EBNA-LP (transcriptional activators/regulators) as well as latent membrane protein 1 (LMP1), LMP2A, and LMP2B (involved in signaling). Lower expression levels are demonstrated by EBV-encoded small non-coding RNAs (ncRNAs) and two clusters of microRNAs (the so-called BHRF1 and BART miRNAs). Type II latency characterized by expression of EBNA-1, LMP1, LMP2A/2B as well as EBER and BART miRNAs is typical of HL and NPC. Finally, in EBV-positive BL cells, type I latency is characterized by expression of EBNA-1, EBER, and BART miRNAs.

Based on the genetic polymorphism in *EBNA-2*, *-3A*, *-3B,* and *-3C* genes, two major strains of EBV, also known as types, were identified: EBV type 1 (EBV-1) and EBV type 1 (EBV-2) [6]. The results of multiple studies show that they differ in their geographic distribution [7]. Most importantly, they differ by their transforming activity *in vitro*. EBV-1 readily transforms B cells in tissue culture, leading to proliferation of immortalized LCLs, while EBV-2 is poorly transforming [6, 8]. These differences in the ability to immortalize B cells *in vitro* lead to the assumption that *in vivo* these virus types use alternative approaches to establish latency. There are data showing that EBV-2, unlike EBV-1, displays a unique tropism for T cells, strongly suggesting that such tropism is a natural part of the EBV-2 life cycle [8]. The paradox of EBV-2 is that although infection of B cells in culture does not cause proliferation of LCLs, EBV-2 is equally detected in endemic BL [9]. Therefore, inability to transform B cells *in vitro* does not imply that this virus type lacks oncogenic potential *in vivo*.

Experimental studies have demonstrated that among more than 85 EBV genes, *LMP1* has the highest transforming and oncogenic potential, thus being seen as one of the major (classical) oncogenes of the virus [10]. The same-name protein encoded by this gene consists of 356 amino acids and includes a short cytoplasmic N-terminal domain, 6 transmembrane domains and a long cytoplasmic C-terminal domain [11]. LMP1 promotes cell proliferation, inhibits apoptosis, increases the mobility of different types of cells *in vitro,* transforms rodent (*Rodentia*) fibroblasts and is essential for transformation of human B cells; it can also induce hyperplasia and lymphoma in transgenic mice (*Mus*) [12]. As the protein polymorphisms are frequently associated with the geographic origin of the virus, Edwards et al. offered a classification in 1999, which is still very popular. The researchers identified several distinct sequence variants of LMP1 by distinguishing signature amino acid changes against the prototype LMP1-B95.8 variant [13]. The variants were named by their geographic location: Alaskan (Ala), China1, -2, and -3 (Chi1, Chi2, Chi3), Mediterranean+ (Med+), Mediterranean– (Med−), and North Carolina (NC). Recent studies have reported new LMP1 variants with unique amino acid changes in EBV strains from Thailand (Thai1 and Thai2), Southeast Asia (SEA1, SEA2), and Argentina (Arg) [14–16].

The identification of new, formerly unknown LMP1 variants suggests that the molecular structure of the virus and its *LMP1* oncogene is associated not only with the geographic regions of its origin, but also with the genetic characteristics of the population (ethnic groups) living in these regions. *In vivo* selection of viral recombinants and/or EBV variants is affected by the immune surveillance and depends on the ability of the pathogen to remain in the host throughout its life cycle. In its turn, the immune surveillance depends on MHC (major histocompatibility complex) types, as functional epitopes undergo modifications depending on their presentation by MHC antigens. Considering that the prevalent types of the latter vary among ethnic groups and populations of different geographic regions, the above factors can be highly important for identification of structural modifications of the virus [17, 18].

The aim of the study was to estimate the EBV infection prevalence and to identify EBV dominant types in ethnic Kalmyks from the Republic of Kalmykia (RK) and ethnic Slavs of the Moscow Region (MR) as a follow-up to the studies on specific features of EBV infection in ancient ethnic groups of the Russian Federation [19]. One of the priorities was to identify prevailing variants of the *LMP1* gene in the strains infecting representatives of the above ethnic groups and to check if there was any relationship between the incidence rates of cancers in the RK and MR population, including EBV-associated tumors, and the EBV type.

Materials and methods

Research materials. The study was performed using mouth washings (MWs) collected from 50 residents of Elista (the capital of RK), who were at least third-generation ethnic Kalmyks. The group consisted of 19 men and 31 women; the mean age was 38.8 years. Similar samples of the biological material were collected from native residents of MR, ethnic Slavs – 21 men and 19 women (the mean age was 34.7 years). Each washing was a cell suspension collected individually from each participant after they rinsed their mouths with 15 ml of sodium chloride solution (0.9% sodium chloride, NaCl) for 30 seconds. The washing samples collected in sealed plastic tubes were stored at 4°C for maximum 2 days prior to the tests. All the participants signed their written informed consent for the participation in the study. The research protocol was approved by the Ethics Committee of the Research Institute of Carcinogenesis, FSBI «National Medical Research Center of Oncology named after N.N. Blokhin» of the Ministry of Health of Russia (Protocol No. 1726 dated March 15, 2022).

DNA extraction and LMP1 gene amplification. The MW cells collected after the centrifugation were used for total DNA isolation by phenol-chloroform deproteinization (extraction). The EBV DNA presence and concentration in the isolated samples were measured by using the real-time polymerase chain reaction (real-time PCR), technique described earlier [19]. The *LMP1* gene was amplified in two stages with outer and inner primers in accordance with the procedure we had used previously [20]. Each PCR product was purified on a spin column (QIAquick PCR Purification kit, cat. 28104, Qiagen, Germany) in accordance with the manufacturer's instruction. The PCR mixture contained ~100–200 ng of the PCR product; the DNA concentration was estimated visually in the agarose gel. DNA extracted from the LMP1-B95.8 cell line (standard) in the amount of 1 µg served as a positive control; water (H_2O) was used as a negative control.

EBV typing. The nested PCR assay was used for detection of nucleic acid of EBV type 1 and type 2. The primers demonstrated high specificity and absence of cross-reaction with the human genome, other viruses or microorganisms [21]. The pairs of primers were as follows:

1st round of amplification – 5'-AGG GAT GCC TGG ACA CAA GA-3' and 5-TGG TGC TGC TGG TGG TGG CAA-3';

2nd round of amplification:

for EBV-1 – 5'-TCT TGA TAG GGA TCC GCT AGG ATA-3' and 5'-ACC GTG GTT CTG GAC TAT CTG GAT C-3';

for EBV-2 – 5'-CAT GGT AGC CTT AGG ACA TA-3' and 5'-AGA CTT AGT TGA TGC TGC CCT AG-3'.

The first round of PCR was performed in 24 µl of the mixture that included: 3 µl of the template; 2.5 µl of the 10x buffer for PCR (pH 8.3); 0.2 µl of DNA polymerase; 0.2 mM of each deoxynucleoside triphosphate of the total volume of 0.2 µl; 0.6 µl of outer primers (at a dilution of 5 pmol per 100 µl) and 17.5 µl of sterile H_2O. The PCR amplification performed in the Mastercycler Personal (Eppendorf, Germany) included an initial denaturation step at 94°C for 5 min, then 30 cycles: denaturation at 94°C for 30 sec, the annealing of the

primers at 60°C for 30 sec; extension at 72°C for 1 min and the final extension step at 72 °C for 5 min.

The second round of amplification was performed in another tube with 3 µl of the PCR product of the first round used as the template. The PCR mixture included 2.5 µl of 10x buffer for PCR (pH 8.3); 0.2 µl of DNA polymerase; 0.2 mM of each deoxynucleoside triphosphate of the total volume of 0.2 µl; 0.6 µl of inner primers (at a dilution of 5 pmol per 100 µl) and 17.9 µl of sterile water. The Mastercycler Personal instrument (Eppendorf) was also used for the PCR amplification. The second round of PCR consisted of 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min 30 sec and the final extension step at 72°C for 7 min.

Purified DNA from the BL Namalwa human cell line was used as a positive control. To detect amplicons, we used electrophoresis using 12 µl of the sample in 1.5% agarose gel containing 1 µg/ml of ethidium bromide ($C_{21}H_{20}BrN_3$). The estimated sizes of target EBV-1 and EBV-2 fragments were 497 and 165 bp, respectively. The PCR testing was repeated twice for each clinical sample.

Quantification of the viral DNA. The number of EBV DNA copies in each MW was measured using real-time PCR, following the procedure described by Lo et al. [22]. To build calibration curves, we used DNAs of the Namalwa cell line, which contained 2 integrated viral genomes; the reference ratio was 3.3 pg of genomic DNA per 1 copy of viral DNA [23]. The reaction process had been described earlier [24].

Sequencing of LMP1 gene PCR products. The *LMP1* amplicons were sequenced in both directions. The sequencing was performed using the ABI PRISM BigDye Terminator v. 3.1 reagent kit (ThermoFisher Scientific, United States); the reaction products were analyzed using the automatic ABI PRISM 3100-Avant DNA sequencer (ThermoFisher Scientific). The data were processed using Chromas 230 and Vector NT software (Invitrogen, United States).

Classification of LMP1 gene samples. The nucleotide sequences of *LMP1* samples amplified from the mouth washings and translated into amino acid sequences were analyzed with reference to the well-known classification offered by Edwards et al. [13]. The classification was based on the sequence analysis of translated sequences of the *LMP1* gene, which were obtained from individuals with EBV-associated diseases and healthy virus carriers from different geographic regions of the world.

Statistical analysis. The number of EBV DNA copies in MWs of the participants was calculated and analyzed using the Mann–Whitney test. The results were presented as median values with an interquartile range (IQR) (the 25th and 75th percentiles). Fisher's exact test was used to calculate exact values of p when comparing the number of the participants infected with EBV type1 or type 2; the differences were considered statistically significant at $p \leq 0.05$. The calculations were made using Statistica software programs for Windows v10.0.

Results

EBV infection prevalence in groups of Kalmyks and Slavs, types of the virus. The study showed that the EBV prevalence rates in MWs of the Kalmyk and Slavic representatives were approximately identical (**Table 1**). The median viral DNA copy number per 1 cell of MW in representatives of each ethnic group was almost equal to zero (M = 0.007 and 0.000, respectively), demonstrating insignificantly different IQRs. These findings suggest that the virus concentration levels in blood of the compared groups also did not differ significantly.

As shown in **Fig. 1**, EBVs of both types in the group of Kalmyks had highly similar distribution (type 1 accounting for 52% and type 2 of the virus accounting for 48%), while in the group of Slavs, EBV-1 dominated over EBV-2 (80.6% compared to 19.4%). The difference between EBV-1 and EBV-2 detection rates in the representatives of both ethnic groups points to the fact that ethnic and, most likely, geographic and other factors can have a significant impact on the

Table 1. The rate of Epstein–Barr virus infection of the oral cavity of the Kalmyks and Slavs
Таблица 1. Инфицированность полости рта вирусом Эпштейна–Барр в группах калмыков и славян

Parameter Показатель	Ethnic Kalmyks, Republic of Kalmykia Этнические калмыки, Республика Калмыкия	Ethnic Slavs, Moscow Region Этнические славяне, Московская область
Number of persons studied, male/female (n) Число обследованных, мужчины/женщины (n)	19/31	15/25
Average age (years) Средний возраст (годы)	38.8	34.7
Number of virus copies per 1 cell oforal cavity wash sample Число копий вируса на 1 клетку смыва полости рта	IQR/МКИ = 0.000–0.184 Median/Медиана = 0.007	IQR/МКИ = 0.000–0.257 Median/Медиана = 0.000
Total Всего	50	40

Note. IQR, intequartile range.

Примечание. МКИ – межквартильный интервал.

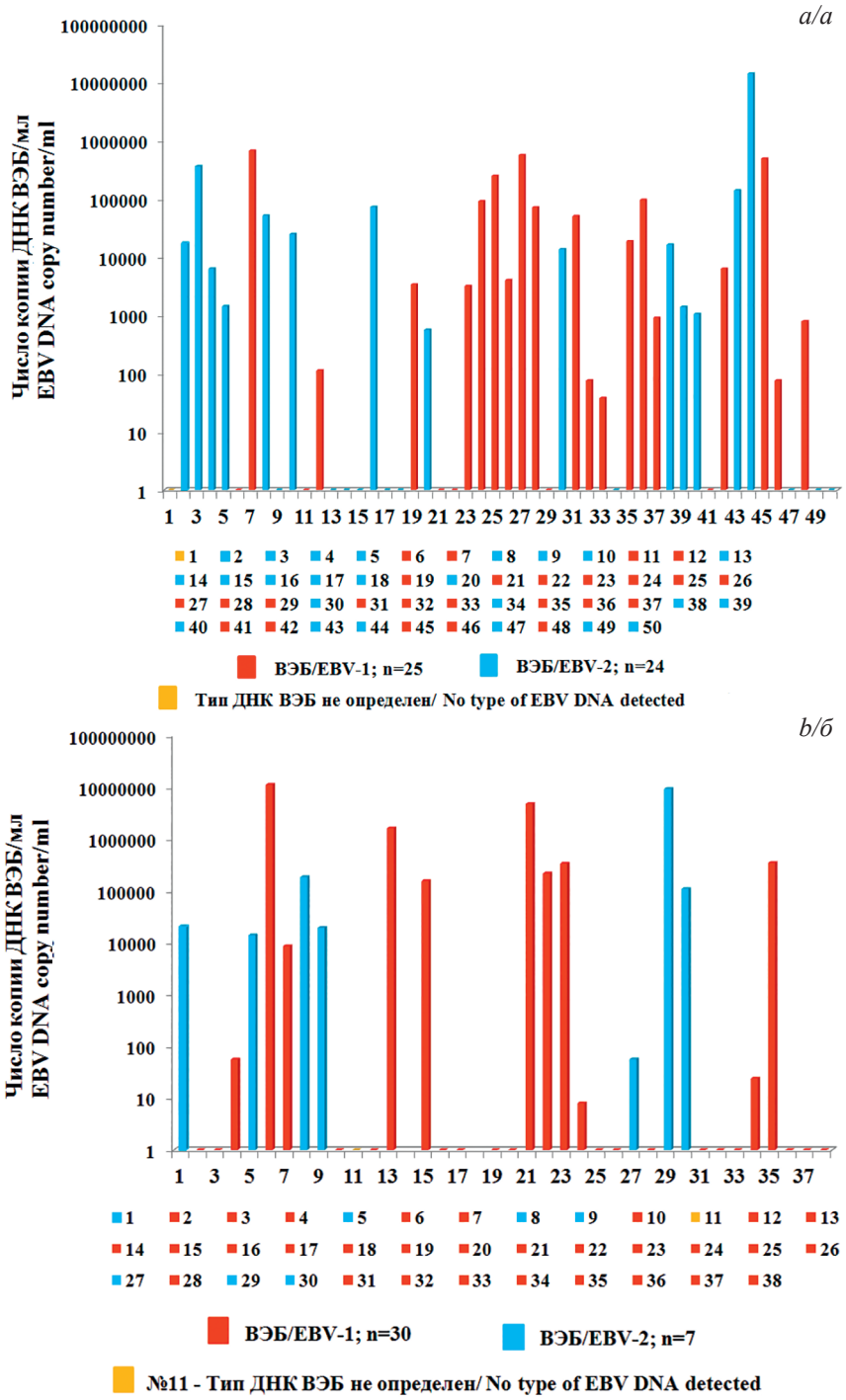

Fig. 1. Epstein–Barr virus types and DNA concentrations per 1 ml of oral cavity wash sample: *a*, in the Kalmyk group; *b*, in the Slavic group.
Рис. 1. Типы вируса Эпштейна–Барр и концентрация ДНК в 1 мл смыва полости рта: *а* – в группе калмыков; *б* – в группе славян.

prevalence and distribution of EBV types in different ethnic populations of the Russian Federation. **Fig. 1** also shows that in the group of Kalmyks, the number of washings containing copies of viral DNA (of both types) was slightly higher compared to the group of Slavs (70.2%; 33/47 against 48.6%; 18/37, respectively). It can be explained by the larger number of virus-containing cells in MW samples in the first case.

EBV LMP1 gene polymorphism in Kalmyks and Slavs. The distribution of *LMP1* gene variants in the virus isolates from the biomaterials of representatives of the Kalmyk and Slavic ethnic groups gives an idea of the polymorphic profile of EBV strains (specifically, in CTAR regions of the studied oncoprotein samples) circulating in the respective populations (**Table 2**). The analysis of nucleotide and deduced amino acid se-

Table 2. *LMP1* gene polymorphism in Epstein–Barr virus isolates from oral cavity wash samples in groups of Kalmyks and Slavs
Таблица 2. Полиморфизм гена *LMP1* в изолятах вируса Эпштейна–Барр из смывов полости рта в группах калмыков и славян

Number of persons studied (*n*) Число обследованных (*n*)	Number of *LMP1* samples (absolute value/ percentage) Число образцов *LMP1* (*n*/%)	*LMP1* variants according to classification of Edwards et al. (1999) [13] out of positive samples Варианты *LMP1* по классификации Edwards и соавт. (1999) [13] из числа положительных образцов					Mutations in CTAR regions of the *LMP1* gene (aa positions) (absolute value/ percentage) Мутации в областях CTAR гена *LMP1* (позиции а.о.) (*n*/%)		
		B95.8	China	Med+	Med–	NC	CTAR1 191–232	CTAR2 351–386	CTAR3 275–330
Ethnic Kalmyks, Republic of Kalmykia Этнические калмыки, Республика Калмыкия									
50	29 (58.0)	22/29 (75.9%)	0/29 (0.0%)	5/29 (17.2%)	1/29 (3.4%)	1/29 (3.4%)	0 (0.0%)	S366A/T: 6/29 (20.7%)	S309T/N: 8/29 (27.6%)
Ethnic Slavs, Moscow region Этнические славяне, Московская область									
40	40 (100.0)	33/40 (82.5%)	3/40 (7.5%)	0/40 (0.0%)	1/40 (2.5%)	3/40 (7.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Note. CTAR, C-terminal activator region. B95.8, China, Med+, Med–, NC are EBV *LMP1* variants.

Примечание. CTAR – С-терминальная активирующая область. B95.8, China, Med+, Med–, NC – варианты *LMP1* ВЭБ.

quences of 29 *LMP1* amplicons obtained from MWs of the Kalmyks (n = 50) and Slavs (n = 40) revealed certain similarities. The *LMP1* samples belonging to its prototype variant LMP1-B95.8 (75.9% and 82.5%, respectively) prevailed in all cases. Both ethnic groups also had other variants of the protein: Med– (3.4% and 2.5%, respectively) and NC (3.4% and 7.5%, respectively). The difference was that the China variant was detected only in the Slavs (75.9%), while Med+ was detected in the Kalmyks. Both variants have the so-called Cao deletion (named after the Cao *LMP1* variant amplified from the tumor tissue of a patient with NPC in the 2000s) and belong to high-transforming variants [25].

The sequence analysis of the amplified *LMP1* samples in the group of Kalmyks detected the Cao-associated S366A/T substitution in CTAR2 (C-terminal activating region) in 6 samples of 29 (20.7%), while the S309T/N substitution was detected in CTAR3 (8 samples of 29). Note that 5 samples from this group of *LMP1* contained the 30-bp deletion (positions 326–355) forming, in its turn, the 10-amino acid deletion typical of the del-LMP1 variant [26]. We will have to significantly expand the observation sampling to measure the extent to which such mutations are typical of the specified population.

Genotyping of EBV isolates in samples from the group of ethnic Kalmyks. The C-terminal domain of LMP1 is known to have three C-terminal activating regions initiating signal transmission: CTAR1 (region 191–232 aa), CTAR2 (351–386 aa) and CTAR3 (275–330 aa) located between them. It has also been found that this domain contains different numbers of repeats (motifs) consisting of 11 amino acids (PQDPDNTDDNG) located between positions 253 and 306 aa. [11, 27]. The prototype variant LMP1-B95.8 contains 4 similar fragments as well as 2 insertions of five amino acids (PHDPL): between the 2nd and 3rd repeats (275–279 aa) and after the last repeat (302–306 aa). The high-transforming Cao-LMP1 variant carries 7 of the above repeats and the insertion of PHDPL, which is also located after the last repeat. The role of changes in the number of repeated sites of PQDPDNTDDNG is still unclear. The insertion consisting of 5 amino acids represents the so-called JAK3-site of CTAR3 (275–330 aa), which assumedly participates in JACK3/STAT-signaling [23, 27].

In 24 (82.3%) cases, the analysis of 29 LMP1 samples from MWs of the ethnic Kalmyks detected a structure composed of a 11-amino-acid fragment and a PHDPL insertion, which was similar to the prototype variant LMP1-B95.8 **(Fig. 2)**.

The other 5 samples contained 7 repeats of 11 amino acids and an insertion of 5 PHDPL fragments after the last repeat. Point (site-specific) mutations D (Asp, aspartic acid) → G (Gly, glycine) in the region of PQDPDNTDDMG repeats were detected in 7 samples.

The obtained data lead to the conclusion that the LMP1 samples from the group of Kalmyks have a structure of repeating elements, which is similar to that of the LMP1-B95.8 variants of the African and Japanese origin [28]. However, compared to Japanese variants of the protein, the LMP1 variants from RK can have point substitutions D→G similarly to LMP1 samples from other Russian isolates [28]. The difference in the number of repeats of the PQDPDNTDDMG fragment and PHDPL insertions (JAK3-motif) is explained by re-combinations occurring during virus replication [27]. There are also hypotheses assuming that mutation changes depend on the geographic origin of EBV strains [29].

Correlation of EBV types with cancer incidence. The analysis of the relationship between the EBV types in the representatives of two ethnic groups and rates of

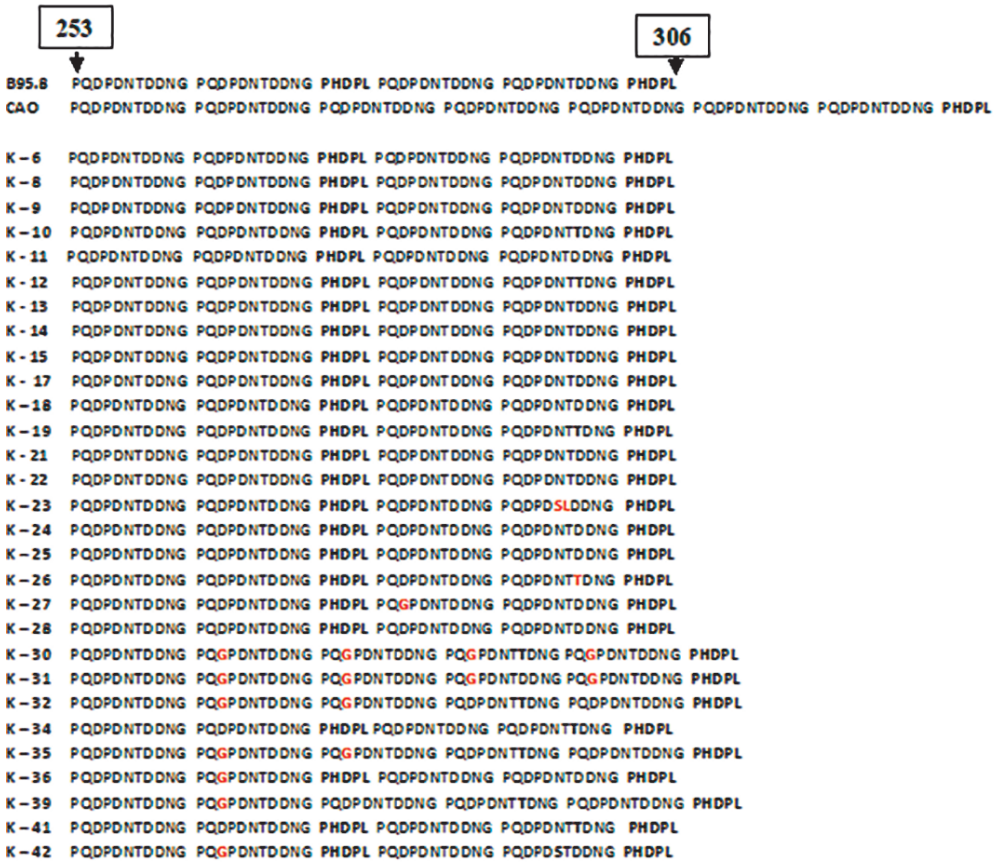

Fig. 2. Variants of 11 amino acid repeat fragments in the C-terminal domain of Epstein–Barr virus *LMP1* gene in isolates from a group of ethnic Kalmyks, Republic of Kalmykia.

Note. B95.8, Cao are variants of the *LMP1* gene; K-6–K-42, coding for *LMP1* isolates from the Kalmyk group; PQDPDNTDDNG, repeat fragment of 11 amino acid residues; PHDPL, insertion of 5 amino acid residues (highlighted in bold); D→G, point (site-specific) mutations in 11 amino acid fragment (positions highlighted in red). The names of the amino acids are given according to the international nomenclature.

Рис. 2. Варианты повторов участков из 11 аминокислот в С-концевом домене изолятов гена *LMP1* вируса Эпштейна–Барр в группе этнических калмыков (Республика Калмыкия).

Примечание. B95.8, Cao – варианты гена *LMP1*; K-6–K-42 – кодовое обозначение изолятов *LMP1* группы калмыков; PQDPDNTDDNG – повторяющиеся группы 11 аминокислот; PHDPL – вставка из 5 последовательностей аминокислот (выделена жирным); D→G – точечные (сайт-специфические) мутации в области фрагмента из 11 аминокислот (позиции выделены красным цветом). Названия аминокислот даны в соответствии с международной номенклатурой.

incidence of malignant tumors of different location showed that (**Fig. 3**) the incidence of gastric cancers and lymphomas in the Slavic population of MR was approximately 2 times as high as that of the RK population.

The dominance of *in vitro* transforming EBV-1 among the ethnic Slavs led to the assumption that the incidence of respective malignancies in MR (affecting a significant proportion of the Slavic population) is higher compared to RK where the Kalmyks accounted for 57.4%, considering that representatives of both ethnic groups were infected with both types of EBV approximately in equal proportions. The incidence rates per 100,000 population of MR for gastric cancer, mouth and throat cancers as well as for malignant lymphomas (including EBV-associated cases) were substantially higher than the rates in the RK population (115, 29, 15, and 97 compared to 68, 26, 7, and 52, respectively); however, the differences were statistically insignificant ($p < 0.05$). The obtained data are consistent with findings of researchers from other countries, which failed to find increased incidence of EBV-associated tumors in the countries with the dominant type 1 virus that is able (as opposed to EBV-2) to transform B cells *in vitro* [30]. Some authors report that the prevalence of type 2 virus characterized by T cell tropism is not also associated with the increased number of EBV-associated diseases among the population [31].

Discussion

EBV is etiologically associated with development of human benign and malignant tumors characterized by diversity and nonuniform distribution across countries. Studies on the polymorphism of genes influencing the biological functions of the virus are aimed to explain geographic differences in the risks of EBV-associated diseases, to identify the role of the virus in their occurrence

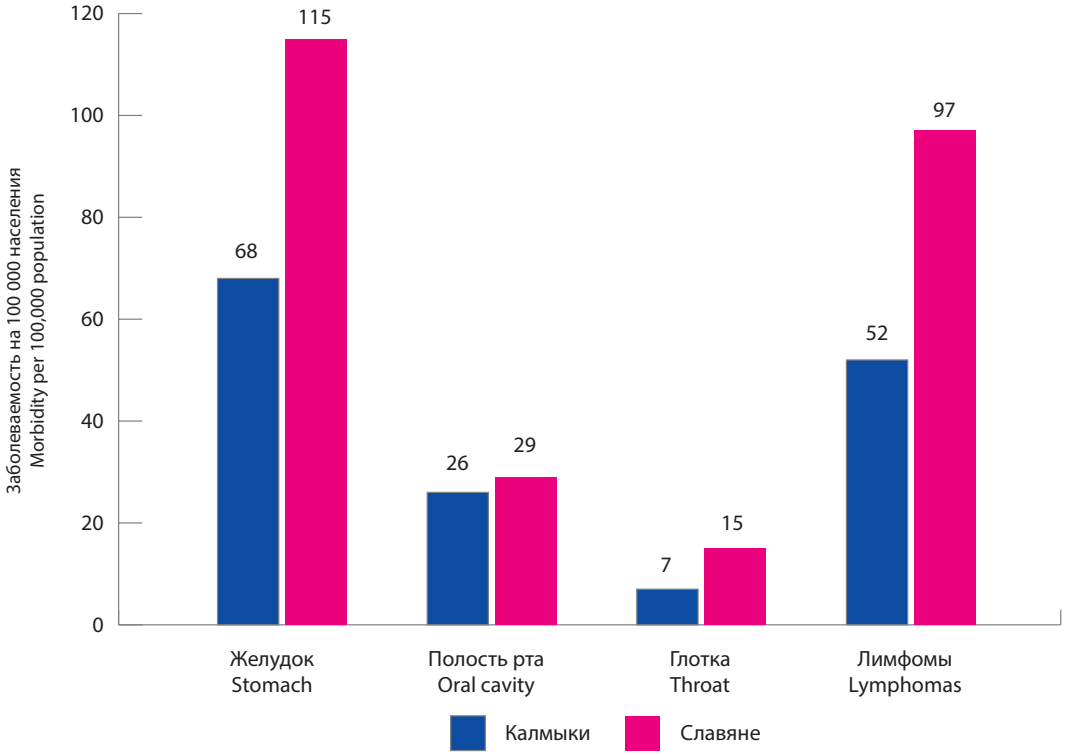

Fig. 3. Incidence of malignant tumors partially associated with Epstein–Barr virus in the population of the Republic of Kalmykia and Moscow Region in 2019.

Рис. 3. Заболеваемость злокачественными опухолями, частично ассоциированными с вирусом Эпштейна–Барр, у населения Республики Калмыкия и Московской области в 2019 г.

and, consequently, to help create an effective vaccine against this virus.

The results of this and previous studies point to the fact that in ethnic groups of the same country (the Russian Federation), both types of EBV can persist in different proportions. For example, our data show that type 2 of the virus is dominant (81%) among the Adygeans living in the Republic of Adygea [32], while in populations of Slavs in MR and Tatars living in the Republic of Tatarstan [19], the dominant type is EBV-1 (81% and 71%, respectively), while in Kalmyks of RK, both types are distributed almost equally (52% and 48%, respectively). The presence and the pattern of infection with EBV of either type, apparently, depend on multiple factors, including the ethnic genotype, which may affect the outcome of infection turning either into chronic virus carriage or EBV-associated pathology.

The results of the study show that the detection rates for either type 1 or type 2 EBV among the representatives of both ethnic groups do not have any effect on cancer incidence rates (including those attributable to EBV-associated cases) in the population representing the respective ethnic groups. The absence of the association is primarily explained by the small (in most situations) number of EBV positive cases among the analyzed malignancies (gastric cancer, mouth and throat cancers, malignant lymphomas), which do not belong to the category of malignancies highly associated with EBV like, for example, NPC. Furthermore, the population of RK and MR consists of representatives of different ethnic groups, with the ethnic Kalmyks and Slavs accounting only for a certain percentage (see the Appendix). Cancer incidence rates for these regions are generalized in terms of the composition of their ethnically heterogenous residents. Therefore, cancer incidence in the total population of the specified regions cannot be associated with any specific type of EBV detected in the selected ethnic representatives. The difference in EBV-1 and EBV-2 distribution among them also did not have any significant effect on the range of the detected variants of the *LMP1* gene or on the structure of repetitive elements of the oncoprotein encoded by the above gene.

Thus, the analysis of the obtained data makes it possible to assume that EBV strains circulating in two ethnic populations have almost identical biological properties. The detected tendency toward increased incidence of gastric cancer, mouth and throat cancers, malignant lymphomas in the population of MR compared to residents of RK is not associated with the dominance of the transforming type of EBV among the representatives of Slavs; rather, this tendency can be explained by genetic susceptibility to the above malignancies among people living in MR. The percentage breakdown of the virus-associated cancer cases in representatives

of specific ethnic groups can be used to confirm or disprove the assumption that EBV types have any effect on development of malignant tumors in the respective populations. In the absence of such data, the above relationship has not been proven yet and needs to be studied further.

Conclusion

The obtained data lead to the conclusion that each of 2 EBV types in different populations can have both dominant and mixed-type distribution, which may be associated with a specific ethnic group, including its genotype. Attention should be also given to other factors, including geographic factors, environmental characteristics of regions. Further studies delving into the prevalence of EBV types in other populations and, first of all, in groups characterized by high frequency of EBV-associated malignancies (for example, NPC), will most likely provide insights into molecular and submolecular mechanisms employed by each type of the virus to initiate and promote carcinogenesis.

REFERENCES

1. Davison A.J., Eberle R., Ehlers B., Hayward G.S., McGeoch D.J., Minson A.C., et al. The order *Herpesvirales*. *Arch. Virol.* 2009; 154(1): 171–7. https://doi.org/10.1007/s00705-008-0278-4
2. Dolan A., Addison C., Gatherer D., Davison A.J., McGeoch D.J. The genome of Epstein–Barr virus type 2 strain AG876. *Virology*. 2006; 350(1): 164–70. https://doi.org/10.1016/j.virol.2006.01.015
3. Young L.S., Rickinson A.B. Epstein–Barr virus: 40 years on. *Nat. Rev. Cancer.* 2004; 4(10): 757–68. https://doi.org/10.1038/nrc1452
4. Moore P.S., Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat. Rev. Cancer.* 2010; 10(12): 878–89. https://doi.org/10.1038/nrc2961
5. McGeoch D.J. Molecular evolution of the *γ–Herpesvirinae*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2001; 356(1408): 421–35. https://doi.org/10.1098/rstb.2000.0775
6. Sample J., Young L., Martin B., Chatman T., Kieff E., Rickinson A., et al. Epstein–Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. *J. Virol.* 1990; 64(9): 4084–92. https://doi.org/10.1128/JVI.64.9.4084-4092.1990
7. Gratama J.W., Ernberg I. Molecular epidemiology of Epstein–Barr virus infection. *Adv. Cancer Res.* 1995; 67: 197–255.
8. Coleman C.B., Wohlford E.M., Smith N.A., King C.A., Ritchie J.A., Baresel P.C., et al. Epstein–Barr virus type 2 latently infects T cells, inducing an atypical activation characterized by expression of lymphotactic cytokines. *J. Virol.* 2015; 89(4): 2301–12. https://doi.org/10.1128/JVI.03001-14
9. Kaymaz Y., Oduor C.I., Yu H., Otieno J.A., Ong'echa J.M., Moormann A.M., et al. Comprehensive transcriptome and mutational profiling of endemic Burkitt lymphoma reveals EBV type-specific differences. *Mol. Cancer Res.* 2017; 15(5): 563–76. https://doi.org/10.1158/1541-7786.MCR-16-0305
10. Bentz G.L., Whitehurst C.B., Pagano J.S. Epstein–Barr virus latent membrane protein 1 (LMP1) C-terminal-activating region 3 contributes to LMP1-mediated cellular migration via its interaction with Ubc9. *J. Virol.* 2011; 85(19): 10144–53. https://doi.org/10.1128/JVI.05035-11
11. Li H.P., Chang Y.S. Epstein–Barr virus latent membrane protein 1: structure and functions. *J. Biomed. Sci.* 2003; 10(5): 490–504. https://doi.org/10.1007/BF02256110
12. Dawson C.W., Port R.J., Young L.S. The role of the EBV-encoded latent membrane proteins LMP1 and LMP2 in the pathogenesis of nasopharyngeal carcinoma (NPC). *Semin. Cancer Biol.* 2012; 22(2): 144–53. https://doi.org/10.1016/j.semcancer.2012.01.004
13. Edwards R.H., Seillier-Moiseiwitsch F., Raab-Traub N. Signature amino acid changes in latent membrane protein 1 distinguish Epstein–Barr virus strains. *Virology*. 1999; 261(1): 79–95. https://doi.org/10.1006/viro.1999.9855
14. Saechan V., Settheetham-Ishida W., Kimura R., Tiwawech D., Mitarnun W., Ishida T. Epstein–Barr virus strains defined by the latent membrane protein 1 sequence characterize Thai ethnic groups. *J. Gen. Virol.* 2010; 91(Pt. 8): 2054–61. https://doi.org/10.1099/vir.0.021105-0
15. Saechan V., Mori A., Mitarnun W., Settheetham-Ishida W., Ishida T. Analysis of LMP1 variants of EBV in Southern Thailand: evidence for strain-associated T-cell tropism and pathogenicity. *J. Clin. Virol.* 2006; 36(2): 119–25. https://doi.org/10.1016/j.jcv.2006.01.018
16. Gantuz M., Lorenzetti M.A., Chabay P.A., Preciado M.V. A novel recombinant variant of latent membrane protein 1 from Epstein Barr virus in Argentina denotes phylogeographical association. *PLoS One.* 2017; 12(3): e0174221. https://doi.org/10.1371/journal.pone.0174221
17. Burrows J.M., Bromham L., Woolfit M., Piganeau G., Tellam J., Connolly G., et al. Selection pressure-driven evolution of the Epstein–Barr virus-encoded oncogene LMP1 in virus isolates from Southeast Asia. *J. Virol.* 2004; 78(13): 7131–7. https://doi.org/10.1128/JVI.78.13.7131-7137.2004
18. Lin H.J., Cherng J.M., Hung M.S., Sayion Y., Lin J.C. Functional assays of HLA A2-restricted epitope variant of latent membrane protein 1 (LMP-1) of Epstein–Barr virus in nasopharyngeal carcinoma of Southern China and Taiwan. *J. Biomed. Sci.* 2005; 12(6): 925–36. https://doi.org/10.1007/s11373-005-9017-y
19. Smirnova K.V., Senyuta N.B., Botezatu I.V., Dushenkina T.E., Lubenskaya A.K., Frolovskaya A.A., et al. Epstein–Barr virus in the ethnic Tatars population: the infection and sequence variants of LMP1 oncogene *[Virus Epshteyna–Barr u etnicheskikh tatar: infitsirovannost' i sikvensnye varianty onkogena LMP1]. Uspekhi molekulyarnoy onkologii.* 2018; 5(3): 65–74. https://doi.org/10.17650/2313-805X-2018-5-3-65-74 (in Russian)
20. Hahn P., Novikova E., Scherback L., Janik C., Pavlish O., Arkhipov V., et al. The LMP1 gene isolated from Russian nasopharyngeal carcinoma has no 30-bp deletion. *Int. J. Cancer.* 2001; 91(6): 815–21. https://doi.org/10.1002/1097-0215(200002)9999:9999<::aid-ijc1122>3.0.co;2-w
21. Salahuddin S., Khan J., Azhar J., Khan J., Muhammad N. Prevalence of Epstein–Barr virus genotypes in Pakistani lymphoma patients. *Asian Pac. J. Cancer Prev.* 2018; 19: 3153–9. https://doi.org/10.31557/APJCP.2018.19.11.3153
22. Lo Y.M., Chan L.Y., Chan A.T., Leung S.F., Lo K.W., Zhang J. Quantitative and temporal correlation between circulating cell-free Epstein–Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. *Cancer Res.* 1999; 59(21): 5452–5.
23. Lawrence J.B., Villnave C.A., Singer R.H. Sensitive, high-resolution chromatin and chromosome mapping in situ: presence and orientation of two closely integrated copies of EBV in a lymphoma line. *Cell*. 1988; 52(1): 51–61. https://doi.org/10.1016/0092-8674(88)90530-2
24. Botezatu I.V., Kondratova V.N., Shelepov V.P., Lichtenstein A.V. DNA melting analysis: application of the "open tube" format for detection of mutant *KRAS*. *Anal. Biochem.* 2011; 419(2): 302–8. https://doi.org/10.1016/j.ab.2011.08.015
25. Smirnova K.V., Diduk S.V., Senyuta N.B., Gurtsevitch V.E.. Molecular biological properties of the Epstein-Barr virus LMP1 gene: structure, function and polymorphism. [*Molekulyarno-biologicheskie svoystva gena LMP1 virusa Epshteyna-Barra: struktura, funktsiya i polimorfizm*] *Voprosi Virusologii*. 2015; 60(3):5-13. (in Russian)
26. Scheinfeld A.G., Nador R.G., Cesarman E., Chadburn A., Knowles D.M. Epstein–Barr virus latent membrane protein-1 oncogene deletion in post-transplantation lymphoproliferative disorders. *Am. J. Pathol.* 1997; 151(3): 805–12.

27. Miller W.E., Edwards R.H., Walling D.M., Raab-Traub N. Sequence variation in the Epstein–Barr virus latent membrane protein 1. *J. Gen. Virol.* 1994; 75(Pt. 10): 2729–40. https://doi.org/10.1099/0022-1317-75-10-2729
28. 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(1): 55–61. https://doi.org/10.1007/s11262-006-0010-y
29. Senyuta N., Yakovleva L., Goncharova E., Scherback L., Diduk S., Smirnova K., et al. Epstein–barr virus latent membrane protein 1 polymorphism in nasopharyngeal carcinoma and other oral cavity tumors in Russia. *J. Med. Virol.* 2014; 86(2): 290–300. https://doi.org/10.1002/jmv.23729
30. Schuster V., Ott G., Seidenspinner S., Kreth H.W. Common Epstein–Barr virus (EBV) type-1 variant strains in both malignant and benign EBV-associated disorders. *Blood.* 1996; 87(4): 1579–85.
31. Khanim F., Yao Q.Y., Niedobitek G., Sihota S., Rickinson A.B., Young L.S. Analysis of Epstein–Barr virus gene polymorphisms in normal donors and in virus-associated tumors from different geographic locations. *Blood*. 1996; 88(9): 3491–501.
32. Lubenskaya A.K., Senyuta N.B., Botezatu I.V., Dushenkina T.E., Liechtenstein A.V., Gurtsevich V.E., Smirnova K.V. Epstein-Barr virus in Adyghes and Slavs in Russia: types of virus, LMP1 variants and malignancies. [*Virus Epshteyna–Barr u adygeytsev i slavyan v Rossii: tipy virusa, varianty LMP1 i zlokachestvennye novoobrazovaniya*] Materialy VI Vserossiyskoy konferentsii po molekulyarnoy onkologii, 21–23 dekabrya 2021 g., Moskva. *Uspekhi molekulyarnoy onkologii*. 2021; 4(8): 96–97. (in Russian)

ЛИТЕРАТУРА

1. Davison A.J., Eberle R., Ehlers B., Hayward G.S., McGeoch D.J., Minson A.C., et al. The order *Herpesvirales*. *Arch. Virol.* 2009; 154(1): 171–7. https://doi.org/10.1007/s00705-008-0278-4
2. Dolan A., Addison C., Gatherer D., Davison A.J., McGeoch D.J. The genome of Epstein–Barr virus type 2 strain AG876. *Virology*. 2006; 350(1): 164–70. https://doi.org/10.1016/j.virol.2006.01.015
3. Young L.S., Rickinson A.B. Epstein–Barr virus: 40 years on. *Nat. Rev. Cancer.* 2004; 4(10): 757–68. https://doi.org/10.1038/nrc1452
4. Moore P.S., Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat. Rev. Cancer.* 2010; 10(12): 878–89. https://doi.org/10.1038/nrc2961
5. McGeoch D.J. Molecular evolution of the *γ–Herpesvirinae*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2001; 356(1408): 421–35. https://doi.org/10.1098/rstb.2000.0775
6. Sample J., Young L., Martin B., Chatman T., Kieff E., Rickinson A., et al. Epstein–Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. *J. Virol.* 1990; 64(9): 4084–92. https://doi.org/10.1128/JVI.64.9.4084-4092.1990
7. Gratama J.W., Ernberg I. Molecular epidemiology of Epstein–Barr virus infection. *Adv. Cancer Res.* 1995; 67: 197–255.
8. Coleman C.B., Wohlford E.M., Smith N.A., King C.A., Ritchie J.A., Baresel P.C., et al. Epstein–Barr virus type 2 latently infects T cells, inducing an atypical activation characterized by expression of lymphotactic cytokines. *J. Virol.* 2015; 89(4): 2301–12. https://doi.org/10.1128/JVI.03001-14
9. Kaymaz Y., Oduor C.I., Yu H., Otieno J.A., Ong'echa J.M., Moormann A.M., et al. Comprehensive transcriptome and mutational profiling of endemic Burkitt lymphoma reveals EBV type-specific differences. *Mol. Cancer Res.* 2017; 15(5): 563–76. https://doi.org/10.1158/1541-7786.MCR-16-0305
10. Bentz G.L., Whitehurst C.B., Pagano J.S. Epstein–Barr virus latent membrane protein 1 (LMP1) C-terminal-activating region 3 contributes to LMP1-mediated cellular migration via its interaction with Ubc9. *J. Virol.* 2011; 85(19): 10144–53. https://doi.org/10.1128/JVI.05035-11
11. Li H.P., Chang Y.S. Epstein–Barr virus latent membrane protein 1: structure and functions. *J. Biomed. Sci.* 2003; 10(5): 490–504. https://doi.org/10.1007/BF02256110
12. Dawson C.W., Port R.J., Young L.S. The role of the EBV-encoded latent membrane proteins LMP1 and LMP2 in the pathogenesis of nasopharyngeal carcinoma (NPC). *Semin. Cancer Biol.* 2012; 22(2): 144–53. https://doi.org/10.1016/j.semcancer.2012.01.004
13. Edwards R.H., Seillier-Moiseiwitsch F., Raab-Traub N. Signature amino acid changes in latent membrane protein 1 distinguish Epstein–Barr virus strains. *Virology*. 1999; 261(1): 79–95. https://doi.org/10.1006/viro.1999.9855
14. Saechan V., Settheetham-Ishida W., Kimura R., Tiwawech D., Mitarnun W., Ishida T. Epstein–Barr virus strains defined by the latent membrane protein 1 sequence characterize Thai ethnic groups. *J. Gen. Virol.* 2010; 91(Pt. 8): 2054–61. https://doi.org/10.1099/vir.0.021105-0
15. Saechan V., Mori A., Mitarnun W., Settheetham-Ishida W., Ishida T. Analysis of LMP1 variants of EBV in Southern Thailand: evidence for strain-associated T-cell tropism and pathogenicity. *J. Clin. Virol.* 2006; 36(2): 119–25. https://doi.org/10.1016/j.jcv.2006.01.018
16. Gantuz M., Lorenzetti M.A., Chabay P.A., Preciado M.V. A novel recombinant variant of latent membrane protein 1 from Epstein Barr virus in Argentina denotes phylogeographical association. *PLoS One.* 2017; 12(3): e0174221. https://doi.org/10.1371/journal.pone.0174221
17. Burrows J.M., Bromham L., Woolfit M., Piganeau G., Tellam J., Connolly G., et al. Selection pressure-driven evolution of the Epstein–Barr virus-encoded oncogene LMP1 in virus isolates from Southeast Asia. *J. Virol.* 2004; 78(13): 7131–7. https://doi.org/10.1128/JVI.78.13.7131-7137.2004
18. Lin H.J., Cherng J.M., Hung M.S., Sayion Y., Lin J.C. Functional assays of HLA A2-restricted epitope variant of latent membrane protein 1 (LMP-1) of Epstein–Barr virus in nasopharyngeal carcinoma of Southern China and Taiwan. *J. Biomed. Sci.* 2005; 12(6): 925–36. https://doi.org/10.1007/s11373-005-9017-y
19. Smirnova K.V., Senyuta N.B., Botezatu I.V., Dushenkina T.E., Lubenskaya A.K., Frolovskaya A.A., et al. Epstein–Barr virus in the ethnic Tatars population: the infection and sequence variants of LMP1 oncogene. *Успехи молекулярной онкологии*. 2018; 5(3): 65–74. https://doi.org/10.17650/2313-805X-2018-5-3-65-74.
20. Hahn P., Novikova E., Scherback L., Janik C., Pavlish O., Arkhipov V., et al. The LMP1 gene isolated from Russian nasopharyngeal carcinoma has no 30-bp deletion. *Int. J. Cancer.* 2001; 91(6): 815–21. https://doi.org/10.1002/1097-0215(200002)9999:9999<::aid-ijc1122>3.0.co;2-w
21. Salahuddin S., Khan J., Azhar J., Khan J., Muhammad N. Prevalence of Epstein–Barr virus genotypes in Pakistani lymphoma patients. *Asian Pac. J. Cancer Prev.* 2018; 19: 3153–9. https://doi.org/10.31557/APJCP.2018.19.11.3153
22. Lo Y.M., Chan L.Y., Chan A.T., Leung S.F., Lo K.W., Zhang J. Quantitative and temporal correlation between circulating cell-free Epstein–Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. *Cancer Res.* 1999; 59(21): 5452–5.
23. Lawrence J.B., Villnave C.A., Singer R.H. Sensitive, high-resolution chromatin and chromosome mapping in situ: presence and orientation of two closely integrated copies of EBV in a lymphoma line. *Cell*. 1988; 52(1): 51–61. https://doi.org/10.1016/0092-8674(88)90530-2
24. Botezatu I.V., Kondratova V.N., Shelepov V.P., Lichtenstein A.V. DNA melting analysis: application of the "open tube" format for detection of mutant *KRAS*. *Anal. Biochem.* 2011; 419(2): 302–8. https://doi.org/10.1016/j.ab.2011.08.015
25. Смирнова К.В., Дидук С.В., Сенюта Н.Б., Гурцевич В.Э. Молекулярно-биологические свойства гена LMP1 вируса Эпштейна–Барр: структура, функции и полиморфизм. *Вопросы вирусологии*. 2015; 60(3): 5–13.

26. Scheinfeld A.G., Nador R.G., Cesarman E., Chadburn A., Knowles D.M. Epstein–Barr virus latent membrane protein-1 oncogene deletion in post-transplantation lymphoproliferative disorders. *Am. J. Pathol.* 1997; 151(3): 805–12.
27. Miller W.E., Edwards R.H., Walling D.M., Raab-Traub N. Sequence variation in the Epstein–Barr virus latent membrane protein 1. *J. Gen. Virol.* 1994; 75(Pt. 10): 2729–40. https://doi.org/10.1099/0022-1317-75-10-2729
28. 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(1): 55–61. https://doi.org/10.1007/s11262-006-0010-y
29. Senyuta N., Yakovleva L., Goncharova E., Scherback L., Diduk S., Smirnova K., et al. Epstein–barr virus latent membrane protein 1 polymorphism in nasopharyngeal carcinoma and other oral cavity tumors in Russia. *J. Med. Virol.* 2014; 86(2): 290–300. https://doi.org/10.1002/jmv.23729
30. Schuster V., Ott G., Seidenspinner S., Kreth H.W. Common Epstein–Barr virus (EBV) type-1 variant strains in both malignant and benign EBV-associated disorders. *Blood.* 1996; 87(4): 1579–85.
31. Khanim F., Yao Q.Y., Niedobitek G., Sihota S., Rickinson A.B., Young L.S. Analysis of Epstein–Barr virus gene polymorphisms in normal donors and in virus-associated tumors from different geographic locations. *Blood*. 1996; 88(9): 3491–501.
32. Лубенская А.К., Сенюта Н.Б., Ботезату И.В., Душенькина Т.Е., Лихтенштейн А.В., Гурцевич В.Э., Смирнова К.В. Вирус Эпштейна–Барр у адыгейцев и славян в России: типы вируса, варианты LMP1 и злокачественные новообразования. Материалы VI Всероссийской конференции по молекулярной онкологии, 21–23 декабря 2021 г., Москва. *Успехи молекулярной онкологии*. 2021; 4(8): 96–97.