NASOPHARYNGEAL CARCINOMA IN INDONESIA HAS A LOW PREVALENCE OF THE 30-BASE PAIR DELETION OF EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1

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Abstract. Epstein-Barr virus (EBV) is associated with nasopharyngeal carcinoma (NPC), one of the highest incidence of tumors in Indonesia. EBV infection is ubiquitous around the world, but NPC occurs with a remarkable geographic distribution. This phenomenon suggests that there are subtypes of EBV, some of which may have greater tumorigenic potential. The latent membrane protein 1 (LMP 1) gene encoded by EBV is tumorigenic due to its ability to transform rodent fibroblast. It was originally shown that the LMP 1 gene from NPC of Chinese patients harbors a deletion of 30-bp in the carboxyl terminal of the gene. However, the deletion is also present in healthy control and in other EBV-positive tumors. We examined the polymorphism of LMP 1 in 56 tumor biopsies of Indonesian patients with NPC and identified low prevalence of the 30-bp deletion of LMP 1. Sequence analysis showed unique mutations of LMP 1 which suggests that strain-specific variations of EBV are found in Indonesia. The low frequency of 30-bp deletion in the country with high prevalence of NPC indicates that the deletion may represent a geographic polymorphism rather than a predisposing factor in the development of NPC.

INTRODUCTION

The human γ herpes virus Epstein-Barr virus (EBV) is an oncogenic DNA virus associated with nasopharyngeal carcinoma (NPC), Burkit lymphoma, Hodgkin's disease and infectious mononucleosis (Kieff, 1996). NPC occurs with a remarkable geographic pattern of incidence. Although incidence is low in Europe and North America, Japan and India, NPC is found with high incidence in southern part of China, Hong Kong, Alaska and Greenland (Muir, 1971; De The, 1982; Lanier *et al*, 1980). The disease has an intermediate incidence rate in northern China, northern Africa (Altun *et al*, 1995; Muir *et al*, 1987). In Indonesia, NPC is the 5th of the most frequent cancers among males and females; in 1999, it contributed about 5.78 % of all cancers.

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EBV can be classified into 2 major strains, type A and type B, based on differences in coding-region sequences in EBNA2, EBNA3A, B, and C antigens and in EBER small polyadenylated RNA sequences (Arrand *et al*, 1989; Rowe *et al*, 1989). Type C variants, which lacks the Bam HI site between the Bam HI WI and I region and type f variant, which has an extra Bam HI site in the Bam HI fragment, have been reported to be associated with NPC (Lung *et al*, 1991).

LMP 1 is an integral type III membrane protein of EBV. It contains 23 amino acids in amino-terminal cytoplasmic domain, six hydrophobic transmembrane domains and 200 amino acids in carboxyl terminal domain (Young *et al*, 1988). LMP 1 was detected in at least 65% of NPC biopsies (Young *et al*, 1988; Liebowitz, 1994). LMP 1, encoded by the EBV-BNLF1 gene, is the transforming protein of EBV, and

induces B-lymphocyte and rodent fibroblast transformation as well as tumor in nude mice (Young, *et al* 1988; Liebowitz, 1994; Baichwal and Sugden, 1988; Wilson *et al*, 1990). LMP 1 induces activation of several B-cell antigens and adhesion molecules, and protects infected cells from apoptosis by up-regulation of the bcl-2 and A-20 genes (Kieff *et al*, 1996). It also interacts with the TRAF signal transduction pathway, resulting in the induction of EGFR expression and activation of NF-κB, AP-1 and JAK/STAT pathways (Hammarskjlold and Simurda, 1992; Miller *et al*, 1995; Mosialos *et al*, 1995; Kieser *et al*, 1997; Gires *et al*, 1999).

Attention has focused on a deleted variant of LMP 1 that was originally identified in tumors of Chinese patients with undifferentiated NPC and is characterized by 30-bp deletion corresponding to codon 343 to 352 of the B95-8 LMP 1, together with other hot spots of point mutations (Chen *et al*, 1992; Hu *et al*, 1991; Sandvej *et al*, 1994; Knecht *et al*, 1995). The geographic distribution of this variant means that the majority of Chinese NPC patients are infected with EBV carrying the deleted-LMP 1 gene (Young *et al*, 1988). In addition, the two prototype deleted-LMP 1 variants, Chinese nasopharyngeal carcinoma (CAO) and 1510, are widely accepted as being more oncogenic than B95-8 gene in rodent fibroblast and a human epithelial cell line (Chen *et al*, 1992; Hu *et al*, 1993; Zheng *et al*, 1994), although these results have been questioned (Nicholson *et al*, 1997).

The incidence of different EBV-associated tumors shows considerable geographical variations. The predominant EBV strain in China, designated as China 1, is characterized by a cluster of 13 nucleotide changes with respect to the B95-8 prototype strain in the amino terminal region of LMP 1 (Miller *et al*, 1994). These include a point mutation resulting in the loss of an Xho1 restriction site. The China1 strain is also distinguished by changes in the carboxyl terminal region of LMP 1, most notably the deletion of amino acids 343-352 (Chen *et al*, 1992; Hu *et al*, 1991; Miller *et al*, 1994; Abdel-Hamid *et al*, 1992). A related strain, previously found in Alaskan isolates, shares 14 of the 15 amino terminal changes with China1, including the Xho1 polymorphism, but at the carboxyl terminus retains amino acids 343-352 and harbors 15 additional nucleotide changes not found in China1 (Miller *et al*, 1994).

Our present study was undertaken to isolate the EBV gene of NPC patients from Malang, Indonesia, and to investigate the incidence of EBV, EBV typing, LMP 1 deletion and to sequence parts of the LMP 1 gene to determine whether additional specific base alterations were present in the gene where EBV typing had not been done.

MATERIALS AND METHODS

Patient tissue specimens

Fifty-six samples of NPC paraffin embedded tissues were obtained from patients at Department of Otolaryngology of the Syaiful Anwar Hospital, Malang, Indonesia. Twenty 10 μm slices were collected in 1.5 ml eppendorf tubes. Samples then were deparaffinized by 30 minutes incubation in 1 ml of xylene (Wako) at room temperature. The pellet was collected by centrifugation for 5 minutes, at 15,000 rpm at 25ºC. After repeating twice, the pellet was washed twice with 70% ethanol. The pellets then were dried. A 400-500 μl aliquot of digestion buffer (50 mM Tris (pH 8.5), 1 mM EDTA, 0.5% Tween 20) containing 100 μg/ml proteinase K (Boehringer Manheim) were added to each tube. After 3 hours of incubation at 5ºC, followed by incubation at 95ºC for 10 minutes, samples were then extracted with phenol-chloroform. DNA was precipitated with the addition of 40 μl of sodium acetate and 1 ml of ethanol then eppendorfs tubes were kept at -80ºC for 15 minutes. DNA was recovered by centrifugation at 15,000 rpm for 15 minutes at 4ºC, washed with 70% ethanol and suspended in 20 μl of Tris-EDTA buffer.

PCR studies for detection of EBV

A set of primers termed EBV-1 (5′-TCC TCG TCC AGC AAG AAG AG 3′) and EBV-2 (5′ CAA CTT GAG GCA GCC TAA TCC 3′) was used to amplify the EBV B-95-8 genome.

The reaction was performed with 200-500 ng of DNA in a 50 μl mixture containing 10 pmol of each primer, 0.2 mM each of the 4 types of deoxynucleotise triphosphate (Takara), and 2.5 units of Taq polymerase (Takara). After initial denaturation at 94ºC for 5 minutes, 30 cycles were performed as follows: 94ºC for 1 minute, 70ºC for 1 minute, and 72ºC for 1 minute. A final 7 minutes at 72ºC completed the PCR amplification. The amplification products were electrophoresed in 3% agarose gel with 100-bp marker and visualized by ethidium bromide staining.

EBNA typing

Two 20-base oligonucleotide primers: flanking a region of the EBNA-2 differing between type A and type B EBV were used, as previously reported (5′-AGGCTGCCCACCC TGAGGAT 3′) and 5′-GCCACCTGGCAGCCC TAAAG 3′) (Lin *et al*, 1993). The reaction was carried out as described above, but using 35 cycles with an annealing temperature of 56ºC. Products were resolved on 8% polyacrylamide gel and visualized by ethidium bromide staining. The expected amplification products of this primer pairs were 168-bp for EBV type A and 184-bp for EBV type B.

LMP 1 deletion analysis

Genomic DNA from each sample was subjected to PCR amplification using two 20-base base oligonucleotide primers flanking the site of the characteristic 30-bp deletion of LMP 1, termed LMP 1-F (5′ CGG AAG AGG TGG AAA ACA AA 3′) and LMP 1-R (5′ GTG GGG GTC GTC ATC ATC TC-3′) as previously published (Vasef *et al*, 1995). The reaction was performed as described above, except that 30 cycles at annealing temperature of 61ºC were employed. In samples that contained or were missing the amino acids 343-352, PCR yielded 181-bp and 151-bp products, respectively. Products were resolved on 3% agarose gel and visualized by ethidium bromide staining.

DNA sequencing

The DNA sequence corresponding to the amino terminal portion of LMP 1 was determined first by amplifying 1.5 ng of genomic DNA with the primer pairs of F3 (5′AAG GAA CAA TGC CTG TCC GTG 3′) and R3 (5′TCT GTC CAC TTG GAG CCC TTT G 3′) and of F4 (5′GGG GCA AAG GGT GTA ATA CTT ACTC 3′) and R4 (5′CAT CGT TAT GAG TGA CTG GAC TGG 3′). PCR products were resolved on 3% agarose gel and visualized by ethidium bromide staining. Amplification products of F3-R3, F4-R4, and LMP 1-F-LMP 1-R primer pairs were excised from the gel. After gel purification (QIAGEN), DNA was subcloned into PT7 blue vector (Novagen, Madison, WI). One clone from each isolate was selected for sequencing. Sequences were determined using a Thermo Sequenase TM II dye terminator cycle sequencing kit (Amersham Life Science) with an automatic DNA sequencer (ABI PrismTM 310 Genetic Analyzer, PE Applied Biosystems).

RESULTS

Fifty-six specimens from patients with NPC were examined and DNA of EBV was isolated. The age of the patients and histological classification of NPC are shown in Table 1. The ages of the patients ranged from 19 to 80 years with median age of 46 years. The histological type of undifferentiated carcinoma was identified in 87.5% of the specimens. We detected EBV gene by PCR in 55 among 56 patients samples, indicating that 98% of NPC we examined had an association with EBV (Fig 1). To determine the EBV type, PCR amplification of the EBNA2 gene was performed. All positive samples showed EBV type A. Type B was not identified in our samples, indicating that EBV in Indonesian NPC was type A (Fig 3). LMP 1 gene was found by PCR in 100% of EBV-positive samples. The 30-bp LMP 1 deletion variant was identified in 25.5% of the specimens, wild type undeleted in 60%, and both the deletion and wild type undeleted LMP 1 gene in 14.5% (Fig 2). The EBV-negative sample did not produce any DNA bands after PCR in these three kinds of studies. The results of EBV prevalence, EBV typing, and presence of LMP 1 deletion are shown in Table 2.

Table 1
Patients classification according to sex, age and histological type of NPC.

	Patients number
Sex	
Male	36
Female	20
Age (year)	
≤30	8
>30	48
Histological type	
Undifferentiated Ca	49
Differentiated Ca	6
Unclassified	1

Table 2
EBV presence, EBV type, and 30-bp LMP 1 deletion in NPC samples.

	Number
EBV positive	55
EBV negative	1
EBV type A	55
EBV type B	0
LMP 1 30-bp deletion	14
LMP 1 30-bp non deletion	33
LMP 1 dual infection	8

Table 3
Sequence polymorphisms in patients samples compared with EBV B95-8 region 168209-168320.

Coordinate in B95-8	Base change	aa change	Frequency
168221	T>C	NC	1/55
168222	T>C	S>P	2/55
168225	T>A	S>T	**42/55**
168238	G>A	NC	2/55
168239	C>T	T>M	2/55
168248	A>C	H>P	13/55
168251	C>A	P>Q	2/55
168255	G>A	D>N	16/55
168258	G>A	G>S	3/55
168264	G>A	G>S	1/55
168265	T>G	H>Q	1/55
168266	A>G	H>R	2/55
168269	G>C	G>A	1/55
168275	A>T	D>V	1/55
168282	A>G	S>G	15/55
168284	A>G	H>R	4/55
168291	G>A	G>S	1/55
168295	A>T	NC	**35/55**
168308	T>C	L>S	**43/55**
168309	T>C	NC	30/55
168320	A>G	Q>R	14/55
168327	G>C	G>R	1/55
168329	G>A	G>E	6/55
168330	G>C	G>R	6/55

NC = no change

Strain of EBV was determined by sequencing of the LMP 1 gene. In this region, variations in the sequences spanning EBV genome position 169352-169075 and position 168340-168189 of the B95-8 prototype strain are shown in Table 3 and Table 4. Alteration of amino acid located in the transmembrane domain of TT**G**/Leu to TT**T**/Phe at codon 67 was seen in 52 out of 55 specimens, G**C**C/Ala to G**G**C/Gly at codon 82 in 51, **T**GT/Cys to **G**GT/Gly at codon 84 in 52, **A**TA/Ile to **C**TA/Leu at codon 85 in 55, **A**CA/Thr to **C**CA/Pro at codon 98 in 49, A**C**A/Thr to A**G**A/Arg at codon 98 in 36 and **T**CT/Ser to **G**CT/Ala at codon 104 in 51 samples. Silent mutations were identified in codon 51 (GC**C**/Ala to GC**G**/Ala, 54/55 samples), condon 63 (ATA/Ile to AT**T**/Ile, 47/55), condon 65 (AT**A**/Ile to AT**C**/Ile, 53/55) and condon 67 (TT**G**/Leu to **C**TG/Leu, 54/55). Mutations of amino acids located in the carboxy-terminal of cytoplasmic domain were T**T**G/Leu to T**C**G/Ser at codon 338, GG**A**/Gly to GG**T**/Gly at codon 342 and **T**CT/Ser to **A**CT/Thr at codon 366 (42/55 samples).

DISCUSSION

Histology of NPC is subclassified into three major groups according to WHO proposal: keratinizing squamous cell carcinoma, non-keratinizing squamous cell carcinoma, and undifferentiated NPC. It is widely known that most studies in high risks area have found 90-100% association of EBV with undifferentiated

Table 4
Sequence polymorphisms in patients samples compaired with EBV B95-8 region 169096-169328.

Coordinate in B95-8	Base change	aa change (codon)	Frequency
169106	C>T	NC (123)	35/55
169108	C>A	L>I (123)	3/55
169116	T>C	L>P (120)	7/55
169118	G>A	NC (119)	14/55
169132	C>T	P>S (115)	1/55
169136	T>C	NC (113)	2/55
169138	C>A	L>I (113)	1/55
169140	C>A	P>H (112)	3/55
169147	C>A	L>I (110)	1/55
169149	C>A	A>E (109)	30/55
169157	C>G	F>L (106)	2/55
169159	T>A	F>I (106)	1/55
169162	T>C	S>P (105)	1/55
169164	C>G	S>C (104)	1/55
169165	T>G	S>A (104)	**51/55**
169176	C>T	P>L (100)	5/55
169182	C>G	T>R (98)	**36/55**
169183	A>C	T>P (98)	**49/55**
169190	T>C	NC (95)	3/55
169194	C>T	T>I (94)	1/55
169203	A>G	K>R (91)	1/55
169209	T>C	M>T (89)	1/55
169221	T>C	I>T (85)	2/55
169222	A>C	I>L (85)	**55/55**
169225	T>G	C>G (84)	**52/55**
169226	T>C	NC (83)	1/55
169230	C>G	A>G (82)	**51/55**
169232	A>G	NC (81)	1/55
169238	A>G	NC (79)	1/55
169245	T>C	L>P (77)	2/55
169247	T>C	NC (76)	3/55
169265	T>C	NC (70)	2/55
169269	T>C	I>T (69)	1/55
169274	G>T	L>F (67)	**52/55**
169276	T>C	NC (67)	**54/55**
169279	A>G	I>V (66)	4/55
169280	A>C	NC (65)	**53/55**
169285	A>T	I>F (64)	5/55
169286	A>T	NC (63)	**47/55**
169288	A>C	I>L (63)	3/55
169292	G>C	M>I (61)	1/55
169294	A>C	M>L (61)	2/55
169295	C>A	NC (60)	31/55
169298	T>A	NC (58)	1/55
169302	T>C	F>S (58)	1/55
169306	T>G	S>A (57)	32/55
169307	C>T	NC (57)	1/55
169312	C>T	L>F (56)	1/55
169318	C>T	L>F (54)	2/55
169322	C>G	NC (51)	**54/55**

NC = no change

Fig 1–EBV detection by PCR after electrophoresis in 3% agarose gel and visualization with ethidium bromide. Lane 1 contains 100-bp molecular mass marker. Lane 2 is a representative of EBV positive case. Lane 3 is EBV negative case. Lane 4 is positive control.

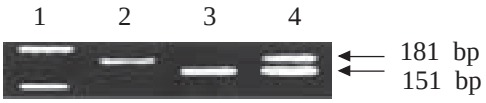

Fig 2–PCR products from PCR amplification of the LMP 1 gene visualized by ethidium bromide staining. The 30-bp deletion shows 151-bp, fragment and undeleted type 181-bp. Lane 1 contains 100-bp molecular mass marker. Lanes 2 is representative of the undeleted case. Lane 3 is representative of the 30-bp deletion case. Lane 4 shows both bands and is representative of dual infection case.

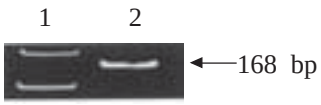

Fig 3–Polyacrylamide gel-electrophoresis of PCR products from amplification of EBV types, visualized by ethidium bromide staining. Type A shows 168-bp and type B 184-bp fragment. All 55 samples (100%) were type A and no type B was detected. Lane 1 contains 100-bp molecular mass marker. Lane 2 is representative of type A.

form and a minor association with differentiated form. While North American forms of NPC demonstrate evidence of differentiation such as keratinization, in Asia NPC is usually undifferentiated and is often with a marked lymphocyte infiltration (Young *et al*, 1988; Liebowitz, 1994). There is a remarkable difference of age distribution between African and Asian NPC. Whereas in South East Asia there is only single peak

of incidence occuring about the age of 50, in North Africa an additional minor peak of incidence appears between the ages of 10-20, comprising about 15% of all NPC patients (Sbih-Lammali *et al*, 1996; Stiller, 1994). In the present study, the median age of NPC patients was 46 years old, with 14% being young patients. There was a high frequency of undifferentiated carcinoma and 98% of the subjects had an association with EBV.

A characteristic 30-bp deletion at the 3′ end of the LMP gene was first identified in NPC in China (Chen *et al*, 1992; Hu *et al*, 1991) and was shown to induce a more aggressive transformation of epithelial cells (Hu *et al*, 1993), although Johnson *et al* (1998) later demonstrated that the 30-bp deletion of EBV LMP 1 is not the major effector of functional differences between variant LMP 1 genes in human lymphocytes, does not inhibit differentiation and induces tumorigenicity of human epithelial cells. Furthermore, it has been proposed that the 30-bp deletion may occur as a result of mispairing of the short repeats flanking the deletion region during replication and the surrounding region, including amino acids 343-352, may constitute a deletion hot spot (Sandvej *et al*, 1994). On the other hand, the C-terminal 30-bp LMP 1 deletion variant has been detected in healthy persons in the endemic regions and can also be found in non endemic areas within Asia (Itakura *et al*, 1996) and Western countries (Sandvej *et al*, 1997) as well as in various EBV-associated lymphoid tumors, including 61% of Danish and 100% of Malaysian peripheral T-cell lymphoma (Sandvej *et al*, 1994), and 80% of Brazilian Burkitt's lymphoma (Chen *et al*, 1996). However, Alaskan (Miller *et al*, 1994) and Russian strains are undeleted, as is the B95-8 prototype strain (Hahn *et al*, 2001). Therefore, it is still unclear whether infection with 30-bp deletion type virus predisposes for the development of NPC. In this study, we found that NPC in Indonesia, which has high frequency of NPC, has a low prevalence of 30-bp deleted-LMP 1. Thus, judging from the results of our study we do not support the notion of an association of LMP 1 deletion with the development of NPC.

In this study we sequenced the LMP 1 gene and identified several nucleotide alterations, which may have an effect on the tumorigenic potential of EBV. Due to lack of samples, we could not sequence the whole LMP 1 gene. The transmembrane domain of LMP 1 is capable of substituting for the ligand-dependent activation of CD-40 and TNF-R2, leading to constitutively activated receptor, and the intact transmembrane domain is necessary for efficient and stable oligomerization (Gires *et al*, 1997). Therefore, the mutations we identified in codons 67, 82, 84, 85, 98 and 104, which are located in the transmembrane domain, may affect the signaling cascade of NF-κB activation by LMP 1. The mutations of codons 338, 342 and 366 located in the C-terminal activating region 2 (CTAR2), which appears to be the sole mediator of JNK-1 and AP-1 activation by LMP 1 (Kieser *et al*, 1997; 1999; Eliopoulus and Young, 1998), and both CTAR1 and 2 are necessary for full activation of NF-κB and efficient B cell immortalization (Kaye *et al*, 1995; Devergne *et al*, 1996; Izumi *et al*, 1997). Those mutations may affect the contribution of LMP 1's ability to transform cells and/or protect B cells from apoptosis in the infection of EBV in NPC. Furthermore, we have identified alterations in nucleotide 169165 (codon 104), 169182 (codon 98), 169183 (codon 98), and 169274 (codon 67), which have not been reported in LMP 1 from China (Sung *et al*, 1998), Alaska (Miller *et al*, 1994), Hong Kong (Cheung *et al*, 1998) and Russia (Hahn *et al*, 2001). These results indicated that specific substrains of EBV have been found in Indonesia.

In summary, we reported that 98% of Indonesian NPC has an association with EBV. One hundred precent was EBV type A, and there was a low frequency of the characteristic 30-bp deleted variant of LMP 1, indicating that the deletion is not a predisposing factor for development of NPC but reflects more the geographic subtypes. We furthermore identified amino acid changes that were different from those reported from other geographic regions, suggesting the existence of specific substrains in Indonesia.

REFERENCES

Abdel-Hamid M, Chen JJ, Constantine N, Massoud M, Raab-Traub N. EBV strain variation: geographical distribution and relation to disease state. *Virology* 1992; 190: 168-75.

Altun M, Fandi A, Dupuis O, Cvitkovic E, Krajina Z, Eschwege F. Undifferentiated nasopharyngeal cancer (UCNT): current diagnostic and therapeutic aspects. *Int J Radiat Oncol Biol Phys* 1995; 32: 859-77.

Arrand JR, Young LS, Tugwood JD. Two families of sequences in the small RNA-encoding region of Epstein-Barr Virus (EBV) correlate with EBV types A and B. *J Virol* 1989; 631: 983-6.

Baichwal VR, Sugden B. Transformation of Balb/3T3 cells by the BNLF1 of Epstein-Barr virus. *Oncogene* 1988; 5: 461-7.

Chen ML, Tsai CN, Liang CL, *et al*. Cloning and characterization of the latent membrane protein (LMP) of a specific Epstein-Barr virus variant derived from the nasopharyngeal carcinoma in Taiwanese population. *Oncogene* 1992; 7: 2131-40.

Chen WG, Chen YY, Bacchi MM, Bacchi CE, Alvarenga M, Weiss LM. Genotyping of Epstein-Barr virus in Brazilian Burkitt's lymphoma and reactive lymphoid tissue: type A with high prevalence of deletion within the latent membrane protein gene. *Am J Pathol* 1996; 148: 17-23.

Cheung ST, Leung SF, Lo KW, *et al*. Specific latent membrane protein gene sequence in type 1 and type 2 Epstein-Barr virus from nasopharyngeal carcinoma in Hong Kong. *Int J Cancer* 1998; 76: 399-406.

De The G. Epidemiology of Epstein-Barr virus and associated diseases in man. In: Roizman B, ed. Herpesvirus. New York: Plenum Press 1982: 25-87.

Devergne O, Hatzivassiliou E, Izumi KM *et al*. Association of TRAF1, TRAF2 and TRAF3 with an Epstein Barr virus LMP 1 domain important for B-lymphocyte transformation: role in NF-κB activation. *Mol Cell Biol* 1996; 16:7098-108.

Eliopoulus AG, Young LS. Activation of cJun N terminal kinase (JNK) pathway by Epstein-Barr virus-encoded latent membrane protein 1 (LMP 1). *Oncogene* 1998; 16: 1731-42.

Gires O, Kohlhuber F, Kilger E, *et al*. Latent membrane protein 1 of Epstein-Barr virus interacts with JAK3 and activates STAT proteins. *EMBO J* 1999; 11: 3064-73.

Gires O, Zimber-Strbl U, Gonella R, *et al*. Latent membrane protein 1 of Epstein-Barr virus mimics a constitutively active receptor molecule. *EMBO J* 1997; 16: 6131-40.

Hahn P, Navikova E, Scherback L, *et al*. The LMP 1 gene isolated from Russian nasopharyngeal carcinoma has no 30-bp deletion. *Int J Cancer* 2001; 9: 815-821.

Hammarskjlold ML, Simurda MC. Epstein-Barr virus latent membrane protein transactivates the human immunodeficiency virus type 1 long terminal repeat through induction of NF-κB activity. *J Virol* 1992; 66: 6496-501.

Hu LF, Chen F, Zheng X, *et al*. Clonability and tumorigenicity of human epithelial cells expressing the EBV encoded membrane protein LMP 1. *Oncogene* 1993; 8: 1575- 83.

Hu LF, Zabarovsky ER, Chen F, *et al*. Isolation and sequencing of the Epstein-Barr Virus BNLF-1 gene (LMP 1) from a Chinese nasopharyngeal carcinoma. *J Gen Virol* 1991; 72: 2399-409.

Itakura O, Yamada S, Narita M, Kikuta H. High prevalence of a 30-base pair deletion and single-base mutations within carboxy terminal end of the LMP 1 oncogene of Epstein-Barr virus in the Japanese population. *Oncogene* 1997; 13: 1549-53.

Izumi KM, Kaye KM, Kieff ED. The Epstein Barr Virus LMP 1 amino acid sequence that engages tumor necrosis factor receptor associated factors is critical for primary B lymphocyte growth transformation. *Proc Natl Acad Sci USA* 1997; 94: 1447-52.

Johnson JR, Stack M, Hazlewood SA, *et al*. The 30-base-pair deletion in Chinese variants of the Epstein-Barr Virus LMP 1 gene is not the major effector of functional differences between variant LMP 1 genes in human lymphocytes. *J Virol* 1998; 72: 4038-48.

Kaye, KM, Izumi KM, Mosialos G, Kieff E. The Epstein Barr Virus LMP 1 cytoplasmic carboxyl terminus is essential for B lymphocyte transformation; fibroblast co-cultivation complements a critical function within the terminal 155 residues. *J Virol* 1995; 69: 675-683.

Kieff, E. Epstein-Barr virus and its replication. In: Fields BN, Knipe DM, Howley PM, *et al*, eds. Virology, 3rd ed. Philadelphia PA: Lippincott-Raven Publisher 1996: 2343-96.

Kieser A, Kaiser C, Hammerschmidt W. LMP 1 signal transduction differs substantially from TNF re-

ceptor 1 signaling in the molecular functions of TRADD and TRAF2. *EMBO J* 1999; 18: 2511-21.

Kieser A, Kilger E, Gires O, Ueffing M, Kolch W, Hammerschmidt W. Epstein- Barr virus latent membrane protein 1 triggers AP1 activity via the c-Jun N- terminal kinase cascade. *EMBO J* 1997; 16: 6478-85.

Knecht H, Bachmann E, Brousset P, *et al*. Mutational hot spots within carboxyl terminal region of the LMP 1 oncogene of Epstein-Barr virus are frequent in lymphoproliverative disorders. *Oncogene* 1995; 10: 523-8.

Lanier A, Bender T, Talbot M. Nasopharyngeal carcinoma in Alaskan Eskimos, Indians, and Aleuts: a review of cases and study of Epstein-Barr virus, HLA, and environmental risk factor. *Cancer* 1980; 46: 2100-06.

Liebowitz D. Nasopharyngeal carcinoma: the Epstein-Barr virus association. *Semin Oncol* 1994; 21: 376-81.

Lin CJ, Lin SC, Chan WP, Evatt BL. Precision of genotyping of Epstein-Barr virus by polymerase chain reaction using three gene loci (EBNA-2, EBNA-3C, and EBER): predominance of type A virus associated with Hodgkin's disease. *Blood* 1993; 81: 3372-81.

Lung ML, Lam WP, Sham J, *et al*. Detection and prevalence of the "f" variant of Epstein-Barr virus in Southern China. *Virology* 1991; 185: 67-71.

Miller WE, Earp HS, Raab-Traub N. The Epstein-Barr virus latent membrane protein 1 induces expression of the epidermal growth factor receptor. *J Virol* 1995; 69: 4390-8.

Miller WE, Edwards RH, Walling DM, Raab-Traub N. Sequence variations in the Epstein-Barr virus latent membrane protein 1 [erratum in *J GenVirol* 1995; 76:1305]. *J Gen Virol* 1994; 75: 2729-40.

Mosialos G, Birkenbach M, Yalamanchili R, Van Arsdale T, Ware C, Kieff E. The Epstein-Barr virus transforming protein LMP 1 engages signaling proteins for the tumor necrosis factor receptor family. *Cell* 1995; 80: 389-99.

Muir, CS. Nasopharyngeal carcinoma in non-Chinese populations with special reference to Southeast Asia and Africa. *Int J Cancer* 1971; 8: 351-63.

Muir CS, Waterhouse J, Mack T, Powell J, Whelan S, eds. Cancer incidence in five continents. Vol V. *IARC Sci Publ* 1987; 88: 1-970.

Nicholson LJ, Hopwood P, Johansen I, *et al*. Epstein-Barr virus latent membrane protein does not inhibit differentiation and induces tumorigenity of human epithelial cells. *Oncogene* 1997; 15: 275-83.

Rowe M, Young LS, Cadwallader K, Petti L, Kieff E, Rickinson AB. Distinction between Epstein-Barr-Virus-type-A (EBNA-2A9) and type-B (EBNA-2B) isolates extends to the EBNA-3 family of nuclear proteins. *J Virol* 1989; 63: 1031-9.

Sandvej K, Gratama JW, Munch M, *et al*. Sequence analysis of the Epsteinm-Barr virus (EBV) latent membrane protein-1 gene and promoter region: identification of 4 variants among wild-type EBV isolates. *Blood* 1997; 90: 323-30.

Sandvej K, Peh SC, Andresen BS, Pallesen G. Identification of potential hot spots in the carboxyl terminal part of the Epstein-Barr Virus (EBV) BNLF-1 gene in both malignant and benign EBV-associated diseases: high frequency of 30-bp deletion in Malaysian and Danish T-cell lymphomas. *Blood* 1994; 84: 4053-60.

Sbih-Lammali F, Djennaoui D, Bclaoui H, Bouguermouh A, Decaussin G, Ooka T. Transcriptional expression of Epstein-Barr virus genes and proto-oncogenes in North African nasopharyngeal carcinoma. *J Med Virol* 1996; 49: 7-14.

Stiller CA. International variations in the incidence of childhood carcinomas. *Cancer Epidemiol Biomark Prev* 1994; 3: 305-10.

Sung NS, Edwards RC, Seillier-Moisewitsch F, Perkins AG, Zheng Y, Traab-Raub N. Epstein-Barr virus strain variation in nasopharyngeal carcinoma from the endemic and non-endemic regions of China. *Int J Cancer* 1998; 76: 207-15.

Vasef MA, Kamel OW, Chen YY, Medeiros LJ, Weiss LM. Detection of Epstein-Barr virus in multiple sites involved by Hodgkin's disease. *Am J Pathol* 1995; 147: 1408-15.

Wilson JB, Weinberg R, Johnson R, Yuspa S, Levine AJ. Expression of the BNLF1 oncogene of Epstein-Barr virus in the skin of transgenic mice induces hyperplasia and aberrant expression of keratin-6. *Cell* 1990; 61: 1315-27.

Young LS, Dawson CW, Clark D, *et al*. Epstein-Barr virus gene expression in nasopharyngeal carcinoma. *J Gen Viro* 1988; 69: 1051-65.

Zheng N, Yuan F, Hu L, Chen F, Klein G, Christensson B. Effect of a B- lymphocyte and NPC derived EBV-LMP 1 gene expression on in vitro growth and differentiation of human epithelial cells. *Int J Cancer* 1994; 57: 747-53.