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Sequence Analysis of the Latent Membrane Protein 1 Genes of Epstein-Barr Virus Isolates in Korea

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The Epstein-Barr virus (EBV)-encoded latent membrane protein 1 (LMP1) is essential for B lymphocyte transformation, and activates NF-κB transcription factor in lymphocytes. LMP1 genes were isolated and sequenced from three type 1 isolates (SNU-321, SNU-538, and SNU-1103) and a type 2 isolate (SNU-20), all derived from Korean cancer patients, to assess sequence variations in the LMP1 of Korean EBV isolates. Sequence analysis revealed that the SNU-1103 and SNU-20 LMP1 genes were nearly identical to that of the prototype B95-8 type 1 EBV strain, with 98% and 96% identities at the nucleotide and protein level, respectively. The SNU-321 and SNU-538 type 1 LMP1 genes both had a G to T substitution at nucleotide position 169,426, resulting in the loss of a *Xho*I site, and a carboxy-termina 30 base pair deletion (position 168,287-168,256), indicating they were variant LMP1 genes, as initially described in a Chinese nasopharyngeal carcinoma-derived EBV isolate (CAO). These two variant LMP1 genes shared more sequence variations than the SNU-20 and SNU-1103 IMP1 geres presumably associated with the LMP1 *Xho*I polymorphism, and showed 96% and 94% sequence identities, respectively, at the nucleotide and amino acid level to respective sequences of B95-8. There were consistent variations between all four isolates and B95-8, including 8-amino acid changes (B95-8 residues 85, 122, 129, 222, 309, 312, 334, 338, and 366) and a 5-amino acid deletion in the carboxy-terminal third 11-amino acid repeat. Transfection of each of these cloned LMP1 genes into Jurkat cells resulted in tenfold stimulation of NF-κB activity, confirming functionality of LMP1 proteins expressed from these genes. Taken together, these results indicate that there is a high degree of overall conservation in sequences of LMP1 between different EBV isolates, yet the distinct sequence variation patterns are consistent with the notion that there are at least two distinct LMP1 variants.

Key words: Epstein-Barr virus, latent membrane protein 1, sequence variation, a 30-bp deleted LMP1 variant

Epstein-Barr virus (EBV) infection of B lymphocytes in vitro results in a latent infection, and infected cells become immortalized or growth-transformed into indefinitely proliferating lymphoblastoid cell lines (LCLs). In LCLs, a limited set of viral gene products, the so-called latent proteins, are actively expressed. The set includes six EBV-encoded nuclear antigens (EBNA1, -2, -3A, -3B, -3C, and -LP) and three latent membrane proteins (LMP1, -2A, and -2B)(7). In addition to the latent proteins, LCLs also show abundant expression of small non-polyadenylated RNAs, EBER1 and 2. Type 1 and type 2 EBV strains coexists in all human populations. The two EBV types maintain characteristic-sequence differences in EBNA2, -3A, -3B, and -3C (6, 32).

LMP1, encoded by the BNLF1 open reading frame of EBV (1), is an integral membrane protein of 386 amino acids, with a short amino-terminal cytoplasmic domain, six hydrophobic membrane-spanning helices, and a long carboxy-terminal cytoplasmic domain. LMP1 is essential for EBV-mediated B cell transformation (15) and is the only EBV latent protein that has oncogenic activity in rodent fibroblast cell lines (2, 26, 38). In EBV-negative B lymphoma cells, LMP1 activates NF-κB (8, 12, 25, 30) and induces most of the changes associated with EBV infection, including the increased expression of activation markers, adhesion molecules, and Bcl-2(9, 39). Expression of LMP1 in epithelial cells causes morphological transformation and inhibits differentiation (11). LMP1 is expressed in most EBV-associated human malignancies, including lymphoproliferative diseases, Hodgkin disease (HD), and nasopharyngeal carcinoma (NPC), suggesting that it may be important in the pathogenesis of these tumors (27). Recent genetic and biochemical analyses of the LMP1 gene have led to a model in which LMP1 is a constitutively activated TNFR(tumor

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necrosis factor receptor)-like membrane receptor that signals through its carboxy-terminal cytoplasmic domain(14, 16). LMP1 aggregates in the membrane without an exogenous ligand. Aggregation, presumably mediated by six hydrophobic transmembrane-spanning domains, is essential for LMP1's transforming activity and NF-κB activation (26).

Sequence analyses of the LMP1 genes in EBV strains (CAO and C1510) in Chinese and Taiwanese NPC specimens have initially defined a variant LMP1 gene, characterized by a 30-base pair (bp) or 10-amino acid deletion in the carboxy-terminal tail and the loss of an *Xho*I site in exon 1 of the gene due to a change from G to T (position, 168,426)(4, 10). Transfection studies have shown that the CAO- and C1510-LMP1 genes are more tumorigenic and less immunogenic than the prototype B958 LMP1 in a murine model system (36). Furthermore, Li *et al.* (23) showed that introduction of the 30-bp deletion into B95-8 LMP1 increases the transforming activity of the protein. These findings have prompted a search of the 30-bp deletion in various EBV associated tumors (13, 17, 18, 19, 20, 22, 24, 33, 34, 36) and have led to claims that the NPC-like LMP1 variant may be associated with aggressive forms of HD (18). However, studies have shown that the LMP1 deletion is also carried in EBV isolates from normal healthy carriers at a frequency similar to that observed in EBV-associated tumors (13, 17). Thus, the role of this deletion in the pathogenesis remains obscure.

Little is known about variations in sequences of the LMP1 genes in EBV strains in Korea. We have recently isolated 17 EBV isolates from a panel of 17 Korean patients with non-EBV-associated cancers (35). Polymerase chain reaction analyses for the *Xho*I site polymorphism and 30-bp deletion in the LMP1 gene revealed that of the 16 isolates analyzed, 11 carry a Chinese NPC-like variant LMP1 gene with the 30-bp deletion and the loss of the *Xho*I site, 2 with the 30-bp deletion only, and 2 without these variations (Cho and Lee, unpublished data). Thus, the results indicate that the 30-bp deleted variant may be prevalent in Korea. In this study, to reveal sequence variations in the LMP1 genes of Korean EBV isolates, the LMP1-coding regions were cloned from 2 type 1 EBV isolates with the NPC-like variant LMP1 gene, and a type 1 and type 2 isolate, both with the prototype B95-8-like LMP1 gene. Their nucleotide and deduced amino acid sequences were compared with those of B95-8 and Chinese NPC EBV isolates.

Materials and Methods

EBV isolates and cell lines

Spontaneous B lymphoblastoid cell lines harboring EBV isolates (SNU-20, SNU-321, SNU-538, and SNU-1103) were derived from Korean cancer patients as previously described by Lee *et al.* (21). SNU-321, SNU-538, and SNU-1103 are EBV-1 isolates and SNU-20 is an EBV-2 isolate. BJAB is an EBV-negative Burkitt's lymphoma cell line. B95-8 is a mammorset B-LCL carrying the prototype B95-8 type 1 strain. Jurkat is a T-leukemia cell line. All cell lines were maintained in RPMI1640 medium (Sigma) supplemented with 10% heat-inactivated FBS (GIBCO) and 2~3 μg/ml of gentamycin (Sigma), and were passaged every 4 days at 3×10^5 cells/ml.

Cloning and sequencing of the LMP1 genes

Polymerase chain reaction was carried out as described by Shim *et al.* (35) to amplify the entire LMP 1-coding regions from cell DNA samples of LCL carrying EBV isolates (SNU-20, SNU-321, SNU-538, and SNU-1103) and B95-8, using Vent polymerase (New England Biolab) and primers that targeted 5'- and 3'-untranslated regions of the LMP1 gene. The primers used were 5'-CGGAATTCCTCAACTGCCTTGCTCCT-3'(B95-8 EBV coordinates, 169,509-169,492) and 5'-CGGGATCCAGTTAAGGTGATTAGCTAA-3' (168,085-168,065). Letters underlined are extra sequences which introduce an *Eco*RI or *Bam*HI recognition site. Amplified LMP1 gene DNAs (1.45 kb) were purified by electrophoresis on a 1% low-melting point agarose gel (FMC, USA) followed by extraction with phenol and with phenol/chloroform (1:1). Purified DNAs were then digested with *Bam*HI and *Eco*RI and cloned into the corresponding sites of pUC19 for sequencing analysis and of pSG5, a SV40 early promoter-based mammalian expression plasmid vector, to yield the expression plasmids for SNU-20 LMP1, SNU-321 LMP1, SNU-538 LMP1, and SNU-1103 LMP1. Sequencing of all cloned LMP 1 genes was carried out using the T7 Sequenase version 2.0 DNA sequencing kit (Amersham Life Science) and α-^{35}S-dATP. Determined sequences were then analyzed using the DNAsis programs.

Transient expression and Western blot assays

The resulting LMP1 expression plasmid (3 μg) was electroporated into 8×10^6 BJAB cells at 200V/960 microfaradays at room temperature using the Bio-Rad Gene Pulser, as previously described (39). Cells were harvested 48 h post-transfection in 400 μl of a lysis buffer containing 40 mM Tris-HCl, pH 7.0, 1.4% SDS, 7% glycerol, and 0.01% bromop-

henolblue. For Western blot assay, proteins in the cell lysates corresponding to 4×10^5 cells were resolved on a 10% SDS-polyacrylamide gel and blotted onto nitrocellulose membrane (Amersham Life Science, England). LMP1 expression was detected by incubation with the anti-LMP1 mAbs CS.1-4 (Novocastra, UK) and then with alkaline phosphatase-conjugated goat anti-mouse IgG (Jackson Laboratory, USA) followed by a standard color reaction using BCIP (5-bromo-4-chloro-3-indolyphosphate) and NBT (nitroblue tetrazolium) (Sigma).

NF-κB activation assay

Jurkat cells (8×10^6) were co-transfected as described above with 3 μg of the LMP1 expression plasmid and 3 μg of a luciferase reporter plasmid, 3X-κB-L or 3X-mutκB-L, along with a β-galactosidase expression plasmid, pHβAPr-1-βgal (5), as an internal control. The two luciferase reporters (25) contain a firefly luciferase gene under a minimal *fos* promoter with three copies of either the histocompatibility complex (MHC) class I κB element (TGGGGATTCCCCA) or its mutated counterpart (TGCGGATTCCCGA). Cells were harvested 48 h after transfection in 250 μl of a reporter lysis buffer (Promega). The luciferase activity was measured in a luminometer (Turner Designs, USA) by mixing 100 μl of luciferase assay substrate (Promega) and 10 μl of the cell lysate, and integrating light release for 15 s. β-Galactosidase activity was determined as previously described by Rosenthal (28). The luciferase activity in each sample was normalized for variations in transfection efficiency represented by the β-galactosidase activity.

Results and Discussion

To reveal sequence variations in the LMP1 genes of Korean type 1 and type 2 EBV strains, the entire LMP1-coding regions were cloned by PCR amplification from three EBV-1 isolates (SNU-321, SNU-538, and SNU-1103) and an EBV-2 isolate (SNU-20), after which their DNA sequences were determined. The complete nucleotide and predicted amino acid sequences of the LMP1 genes from these four isolates are presented in Fig. 1 and 2, and are aligned with the respective LMP1 sequences of the prototype type 1 EBV strain, B95-8.

The B95-8 EBV-1 LMP1 gene consists of three exons which are predicted to encode 386 amino acids (1). Both the SNU-1103 EBV-1 and SNU-20 EBV-2 LMP1 coding sequences were shorter than the B95-8 LMP1 coding sequence by 15-bp and thus would encode a LMP1 protein that is shorter by 5 amino acids. The 15-bp deletion resulted in the absence of a 5-amino acid (aa) unique sequence (HDPLP) that disrupts the third 11-amino acid repeat sequence in the B95-8 LMP1. In addition to the deletion, there were 20 and 21 nucleotide substitutions in SNU-20 and SNU-1103, respectively. Of these changes, 16 including 12 identical changes resulted in amino acid substitutions in both isolates (Fig. 2). Thus, overall, the SNU-1103 type 1 and SNU-20 type 2 LMP1 genes had the same level of nucleotide and amino acid sequence identity (98% and 96%, respectively) relative to the B95-8 counterparts. The results are consistent with a previous report by Sample *et al.* (31) that the sequences of type 1 and 2 EBV strains were nearly identical, unlike the EBNA2 and EBNA3 family genes (32). With the exceptions of a glutamine to glutamic acid substitution at residue 322 and a glutamine to arginine substitution at residue 334 in the carboxy terminal region, the remaining changes were relatively conservative.

Previous PCR analysis (Cho and Lee, manuscript in preparation) indicated that SNU-321 and SNU-538 carried a variant LMP1 gene characterized by loss of an *Xho*I site in the first exon and a carboxy-terminal 30-bp deletion, as originally described in Chinese NPC EBV isolates (4, 10). As expected, the sequence analysis confirmed that the loss of the *Xho*I site was due to a G-to-T mutation at nucleotide position 168,426 and the presence of the carboxy-terminal 30-bp deletion (168,287 to 168,256) (Fig. 1). There was the same 5-bp deletion in the carboxy-terminal 33-bp repeat region that was found in the SNU-20 and SNU-1103 LMP1 genes. However the SNU-321 and SNU-538 LMP1 genes had three and five perfect 33-bp repeats, respectively, compared with four copies in the B95-8 LMP1. Thus, the predicted SNU-321 and SNU-538 LMP1 proteins would be shorter than the B95-8 counterpart by 26 and 4 amino acids, respectively. In addition to these variations, the SNU-321 and SNU-538 LMP1 genes had 43 and 45 nucleotide changes (40 of which were identical), leading to 23 and 25 amino acid substitutions, respectively (Fig. 2). Twenty of the amino acid changes were due to identical nucleotide changes. Overall, the identity between SNU-321 and B95-8 was approximately 96% and 94% at the nucleotide and amino acid level, respectively, and that between SNU-538 and B 95-8 was 96% and 94%. Nonconservative amino acid changes occurred in both isolates at residues 13 (Arg to Pro), 17 (Arg to Leu), 46 (Asp to Asn), 150 (Asp to Ala), and 334 (Gln to Arg). Occasional nonconservative changes were also detected at residues 82 (Cys to Asp in SNU-321; Cys to Gly in

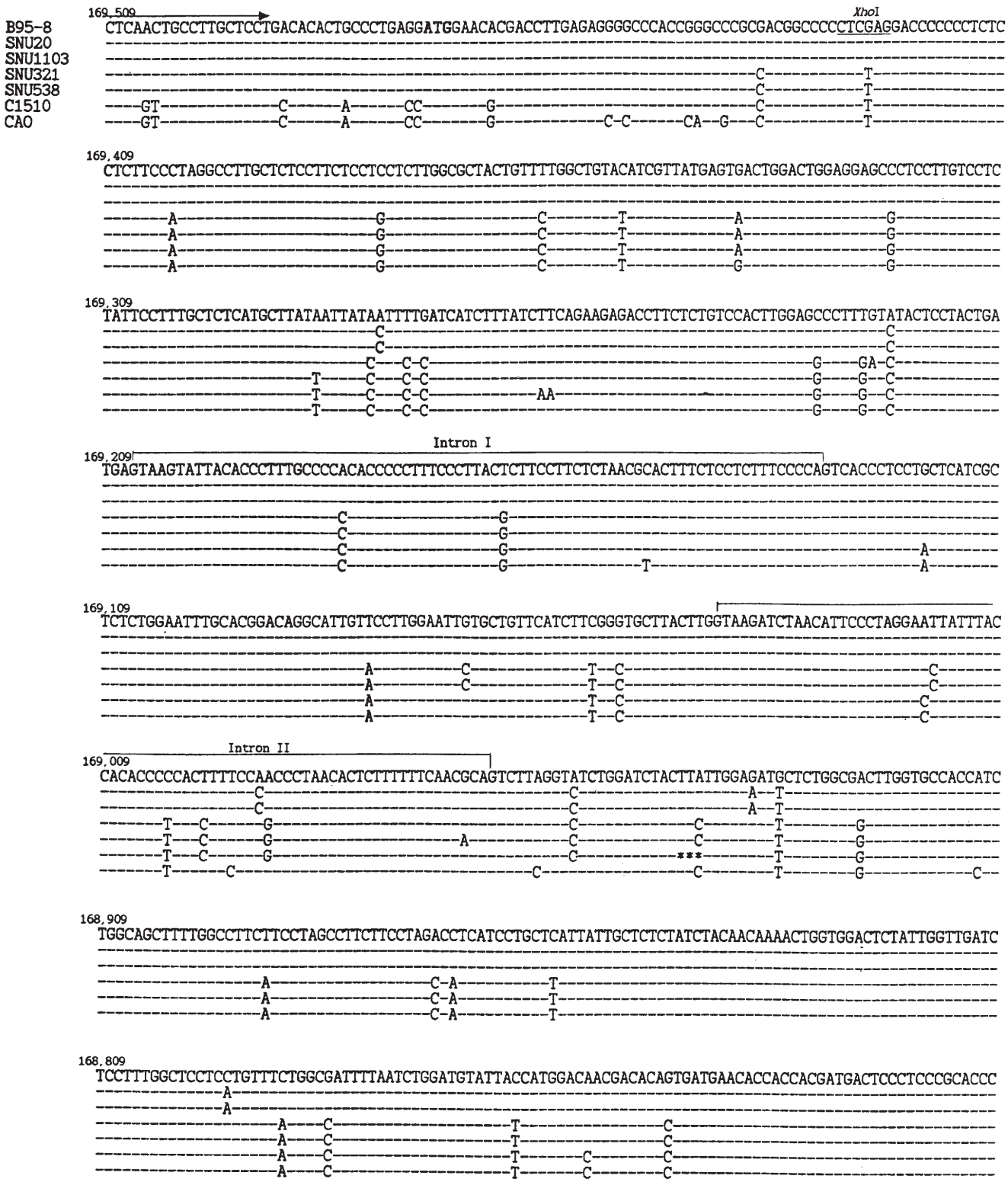

Fig. 1. Alignment of EBV DNA sequences, which enconpass the entire LMP1-coding region, from the SNU-20 type 2 and SNU-321, SNU-538, and SNU-1103 type 1 EBV isolates derived Korean cancer patients and also from CAO Chinese (10) and C1510 Taiwanese NPC isolates (10), with respect to the corresponding sequence of the B95-8 type 1 prototype strain. The primers used for PCR amplification of the above DNA from Korean EBV isolates are indicated by arrows. Letters in bold are the translation initiation and stop codons for the LMP1 gene. A *Xho*I recognition site in exon I of the gene is underlined. Introns are bracketed by lines. Hyphens (–) and asterisks (*) indicate identical and deleted sequences, respectively.

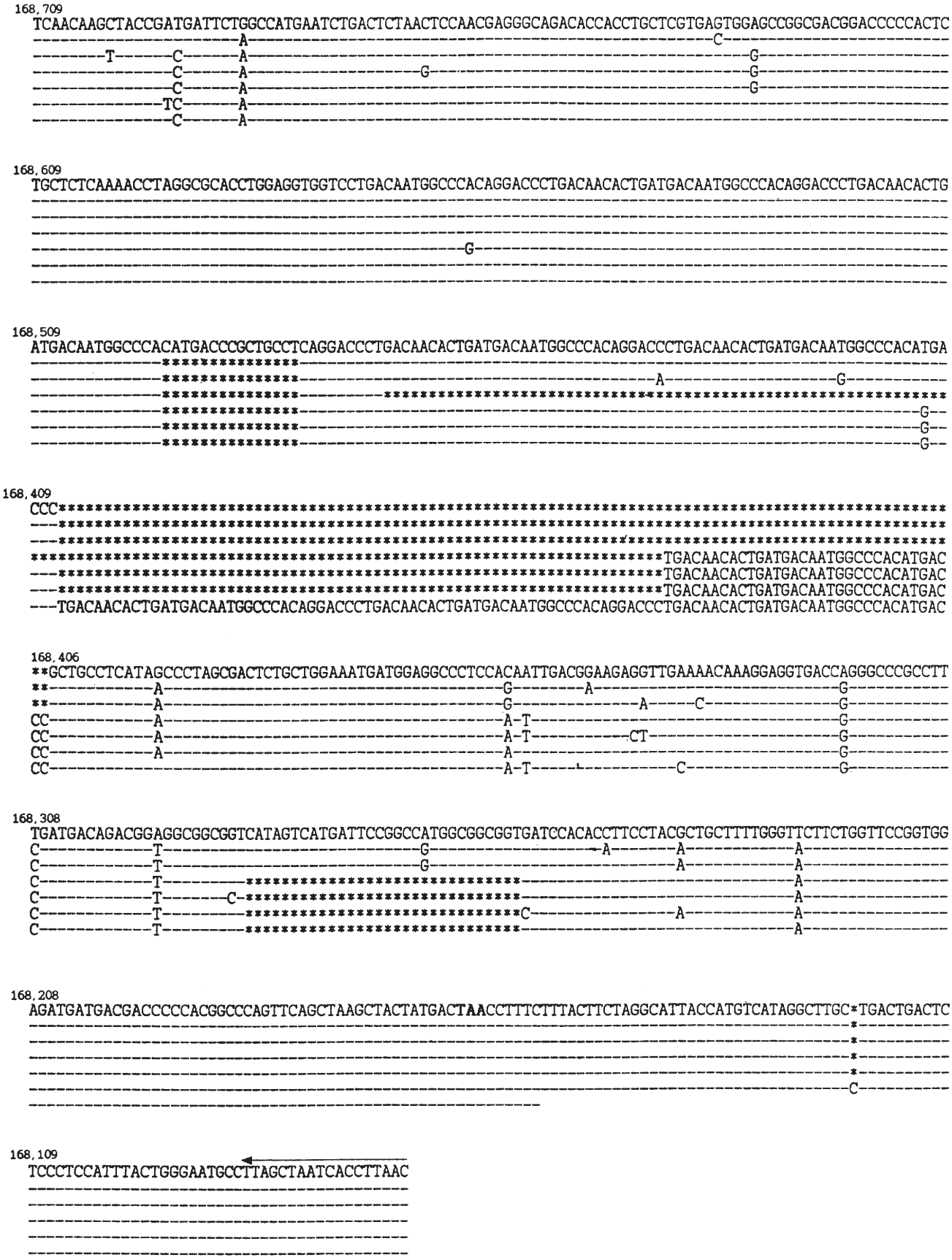

Fig. 1. Continued.

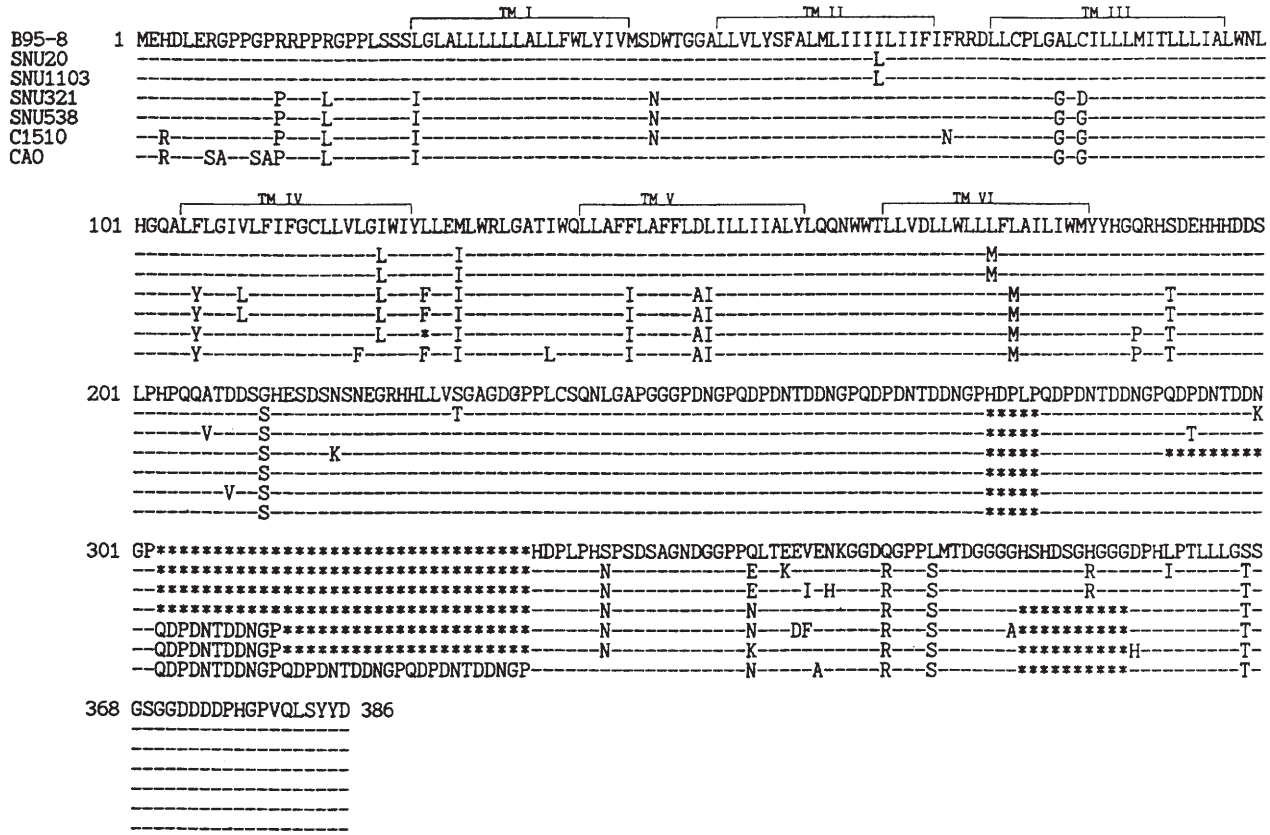

Fig. 2. Alignment of the predicted amino acid sequences of LMP1 from the SNU-20 type 2 and SNU-321, SNU-538, and SNU-1103 type 1 EBV isolates derived from Korean cancer patients and also from CAO Chinese and C1510 Taiwanese NPC isolates, with respect to the B95-8 LMP1. TM1 to TM6 indicate six hydrophobic transmembrane domains of LMP1. Hyphens (–) and asterisks (*) indicate identical and deleted sequences, respectively.

SNU-538) and 218 (Asn to Lys in SNU-321).

Taken together, the sequence analysis revealed that besides the common 5-aa deletion in the 11-aa repeat region, there were consistent variations at residues 85, 122, 129, 222, 309, 312, 334, 338, and 366 in all four EBV isolates (Fig. 2). The same changes have also been present in most Chinese NPC isolates including CAO and C1510 and also European EBV isolates (24, 34), suggesting that these sites may be mutational hot spots. The analysis also revealed that there were a considerable number of additional variations conserved among the LMP1 variants, marked by the loss of the *Xho*I site and the carboxy-terminal 30-bp deletion, such as those of the SNU-321 and SUN-538 type 1 as well as the Chineses NPC-derived CAO and C1510 type 1 isolates. Most of these additional changes clustered within the amino-termina cytoplasmic and hydrophobic transmembrane domains. A similar pattern of LMP1 variations was also observed in European type 1 and Alaska Eskimo NPC type 2 isolates canying the *Xho*I site polymorphism and the 30-bp deletion (24, 34). Therefore, it appears that such LMP1 variations are not EBV type- or geographic-specific, but rather a general feature in wild type EBV isolates. It is possible that nonconservative changes in the transmembrane domains could affect the folding or positioning of LMP1 and thereby could cause subtle changes in interactions between LMP1 and cellular proteins. Previous analysis by Miller *et al.* (24) of a large collection of EBV isolates in associated tumors has indicated that these changes are linked to the loss of the *Xho*I site in exon 1 of the gene rather than to the 30-bp deletion.

Functional assays for LMP1 proteins from cloned genes

To determine if LMP1 genes cloned from the SNU-20, SNU-321, SNU-538, and SNU-1103 isolates were functional at the protein level, these genes were subcloned into the pSG5 expression vector to yield corresponding LMP1 expression plasmids. The resulting plasmids were then electroporated into BJAB cells, an EBV-negative lymphoma cell line, and Western blot assays were carried out using a pool of four LMP1 specific monoclonal antibodies (CS.1-4) which recognize different

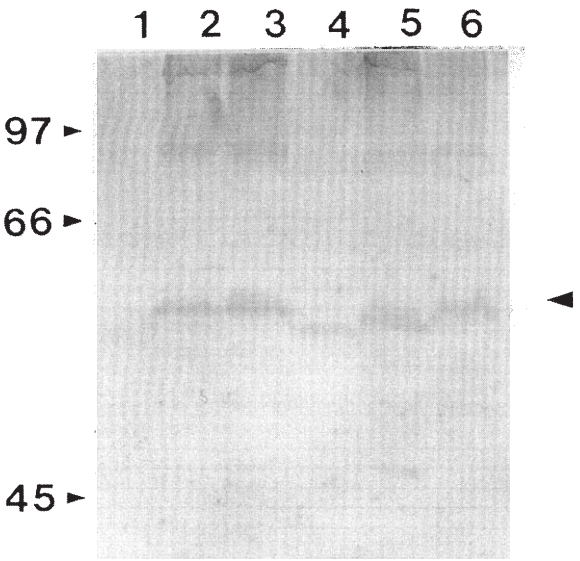

Fig. 3. Western blot assay for expression of LMP1 from cloned SNU-20, SNU-321, SNU-538, and SNU-1103 LMP1 genes. pSG5 expression plasmids containing each of the cloned LMP1 genes from the SNU-20, SNU-321, SNU-538, and SNU-1103 type 1 EBV isolates were electroporated in BJAB cells as described in Materials and Methods. Proteins in the cell lysates prepared 48 h post-transfection were resolved on a 10% SDS polyacrylamide gel and blotted onto a membrane. LMP1 on the membrane was detected using the LMP 1 mAbs CS1.4 as described in Materials and Methods. Lane 1, cells transfected with the pSG5 vector; Lanes 2 to 6, cells with the B95-8, SNU-20, SNU-321, SNU-538, and SNU-1103 LMP1 expression plasmids, respectively.

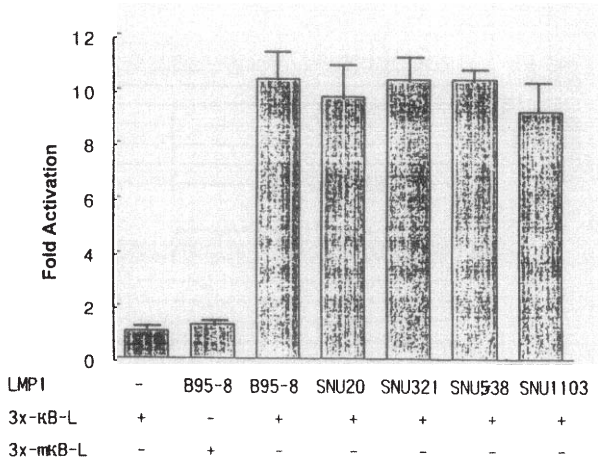

Fig. 4. Assays for NF-κB activation by LMP1 expressed from cloned SNU-20, SNU-321, SNU-538, and SNU-1103 LMP1 genes. Eight million Jurkat cells were co-transfected with 3 μg of the LMP1 expression plasmid as indicated above, 3 μg of 3X-κB-L or 3X-mutκB-L luciferase reporter, and 4 μg of a human b-actin promoter/β-galactosidase reporter as control. The 3X-κB-L and 3X-mutκB-L plasmids contain a firefly luciferase gene and a minimal fos promoter with three copies of either the wild type or altered B element. Cell lysates were prepared 48 h after transfection and analyzed for luciferase and β-galactosidase. The luciferase activities in each of the cell lysates were normalized with the corresponding β-galactosidase activities. Fold activation was determined relative to reporter construct only.

epitopes in the LMP1 carboxy-terminal domain. As shown in Fig. 3, we could detect LMP1 proteins with apparent molecular masses expected from the respective sequence data of the SNU-20, SNU-321, SNU-538, and SNU-1103 genes. Corresponding LMP1 proteins were also detected in similar immunoblot assays for LCLs carrying these EBV isolates (data not shown). We then asked whether expressed LMP1 could activate the NF-κB transcription factor and thus be functional. A luciferase reporter plasmid, 3X-κB-L or 3X-mutκB-L, was used to assay for transcriptional activation by NF-κB. The plasmids contain a minimal fos promoter with three copies of either a wild type κB element or altered counterpart (25). The expression plasmid carrying each of the cloned LMP1 genes or the B95-8 LMP1 gene, as positive control, and the 3X-κB-L luciferase reporter were electroporated into Jurkat cells along with a β-galactosidase expression plasmid as internal control. Luciferase and β-galactosidase activities in the cell lysates were determined 48 hr post-transfection. As shown in Fig. 4, expression of B95-8 LMP1 in Jurkat cells increased luciferase activity tenfold from the 3X-κB-L reporter but not from the 3X-mutκB-L reporter, compared with the levels seen in non-LMP1 expressing cells. Comparable levels of luciferase activity were obtained from the 3X-κB-L reporter by expression of LMP1 proteins from each cloned LMP1 gene isolate. The results thus proved that LMP1 proteins expressed from the cloned LMP1 genes were functional and also indicated that nonconservative amino acid changes and the 30-bp deletion detected in this study do not affect the ability of LMP1 to activate the NF-κB transcription factor.

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References

1. **Baer, R., A.T. Bankier, M.D. Biggin, P.L. Deininger, P.J. Farrell, T.J. Gibson, G. Hatfull, G.S. Hudson, S. C. Satchwell, C. Seguin, P.S. Tuffnell, and B.G. Bar-**

rell. 1984. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. *Nature* **310**, 207-211.
2. **Baichwal, V.R. and B. Sugden.** 1988. Transformation of Balb 3T3 cells by the BNLF-1 gene of Epstein-Barr virus. *Oncogene* **2**, 461-467.
3. **Brodeur, S.R., G. Cheng, D. Baltimore, and D.A. Thorley-Lawson.** 1997 Localization of the major NF-κB-activation site and the sole TRAF3 binding site of LMP-1 defines two distinct signaling motifs. *J. Biol. Chem.* **272**, 19777-19784.
4. **Chen, M.L., C.N. Tsai, C.L. Liang, C.H. Shu, C.R. Huang, D. Sulitzeanu, S.T. Liu, and Y.S. Chang.** 1992. Cloning and characterization of the latent membrane protein (LMP) of a specific Epstein-Barr virus variant derived from the nasopharyngeal carcinoma in the Taiwanese population. *Oncogene* **7**, 2131-2140.
5. **Gunning P, J. Leavitt, G. Muscat, S.Y. Ng, and L. Kedes.** 1987. A human beta-actin expression vector system directs high-level accumulation of antisense transcripts. *Proc. Natl. Acad. Sci. USA* **84**, 4831-4835.
6. **Dambaugh, T., K. Henessy, L. Chamnankit, and E. Kieff.** 1984. U_2 region of Epstein-Barr virus DNA may encode Epstein-Barr nuclear antigen 2. *Proc. Natl. Acad. SCI. USA* **81**, 7632-7636.
7. **Elliott K.** 1996. Epstein-Barr virus and its replication, p. 2343-2396. *In* B.N. Fields, D.M. Knipe, P.M. Howley, R. M. Chanock, J.L. Melnick, T.P. Monath, B. Roizman, and S. Straus. (eds.), Fields Virology, 3rd ed. Raven Press, Philadelphia.
8. **Hammarskjold, M.L. and M.C. Simurda.** 1992. Epstein-Barr virus latent membrane protein transactivates the human immunodeficiency virus type 1 long terminal repeat through induction of NF-kappa B activity. *J. Virol.* **66**, 6496-6501.
9. **Henderson, S., M. Rowe, C. Gregory, D. Croom-Carter, F. Wang, R. Longnecker, E. Kieff, and A. Rickinson.** 1991. Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. *Cell* **65**, 1107-1115.
10. **Hu, L.F., E.R. Zabarovsky, F. Chen, S.L. Cao, I. Ernberg, G. Klein, and G. Winberg.** 1991 Isolation and sequencing of the Epstein-Barr virus BNLF-1 gene (LMP1) from a Chinese nasopharyngeal carcinoma. *J. Gen. Virol.* **72**, 2399-2409.
11. **Hu, L.F., F. Chen, X. Zheng, I. Emberg, S.L. Cao, B. christensson, G. Klein, and G. Winberg.** 1993. Clonability and tumorigenicity of human epithelial cells expressing the EBV encoded membrane protein LMP1. *Oncogene* **8**, 1575-1583.
12. **Huen, D.S., S.A. Henderson, D.C-.Carter, and M. Rowe.** 1995 The Epstein-Barr virus latent membrane protein-1 (LMP1) mediates activation of NF-kB and cell surface phenotype via two effector regions in its carboxy-terminal cytoplasmic domain. *Oncogene* **10**, 549-560.
13. **Itakura, O., S. Yamada, M. Narita, and H. Kikuta.** 1996. High prevalence of a 30-base pair deletion and single-base mutations within the carboxy terminal end of the LMP-1 oncogene of Epstein-Barr virus in the Japanese population. *Oncogene* **13**, 1549-1553.
14. **Izumi, K.M. and E.D. Kieff.** 1997 The Epstein-Barr virus oncogene product latent membrane protein 1 engages the tumor necrosis factor receptor-associated death domain protein to mediate B lymphocyte growth transformation and activate NF-κB *Proc. Natl. Acad. Sci. USA* **94**, 12592-12597.
15. **Kaye, K.M., K.M. Izumi, and E. Kieff.** 1993 Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. *Proc. Natl. Acad. Sci. USA* **90**, 9150-9154.
16. **Kaye, K.M., O. Devergne, J.N. Harada, K.M. Izumi, R. Yalamanchili, E. Kieff, and G. Mosialos.** 1996 Tumor necrosis factor receptor associated factor 2 is a mediator of NF-κB activation by latent infection membrane protein 1, the Epstein-Barr virus transforming protein. *Proc. Natl. Acad. Sci. USA* **93**, 11085-11090.
17. **Khanim, F., Q.Y. Yao, G. Niedobitek, S. Sihota, A.B. Rickinson, and L.S. Young.** 1996 Analysis of Epstein-Barr virus gene polymorphisms in normal donors and in virus-associated tumors from different geographic locations. *Blood* **88**, 3491-3501.
18. **Kingma D.W., W.B. Weiss, E.S. Jaffe, S. Kumar, K. Frekko, and M. Raffeld.** 1996. Epstein-Barr virus latent membrane protein-1 oncogene deletions: Correlation with malignancy in Epstein-Barr virus-associated lymphoproliferative disorders and malignant lymphomas. *Blood* **88**, 242-251.
19. **Knecht, H., E. Bachmann, P. Brousset, K. Sandvej, D. Nadal, F. Bachmann, B. Odermatt, G. Delsol, and G. Pallesen.** 1993. Deletions within the LMP-1 oncogene of Epstein-Barr virus are clustered in Hodgkins disease and identical to those observed in nasopharyngeal carcinoma. *Blood* **82**, 2937-2942.
20. **Knecht, H., M. Raphael, C. McQuain, S. Rothenberger, G. Pihan, S. Camilleri-Broet, E. Bachmann, G.R. Kershaw, S. Ryan, E.L.W. Kittler, P.J. Quesenberry, D. Schlaifer, B.A. Woda, and P. Brousset.** 1996. Deletion variants within the NF-κB activation domain of the LMP1 oncogene prevail in acquired immunodeficiency syndrome-related large cell lymphomas and human immunodeficiency virus-negative atypical lymphoproliferations. *Blood* **87**, 876-881.
21. **Lee, W.K., S.M. Kim, Y.S. Shim, S.G. Cho, S.H. Park, C.W. Kim, and J.-G. Park.** 1998. Characterization of B-lymphoblastoid cell lines established from Korean cancer patients. *In Vitro Cell. Dev. Biol. -AnimalIn* **34**, 97-100.
22. **Knecht, H., E. Bachmann, P. Brousset, S. Rothenberger, H. Einsele, V.S. Lestou, G. Delsol, F. Bachmann, P.F. Ambros, and B.F. odermatt.** 1995. Mutational hot spots within the carboxy terminal region of the LMP1 oncogene of Epstein-Barr virus are frequent in lymphoproliferative disorders. *Oncogene* **10**, 523-528.
23. **Li, S.N., Y.S. chang, and S.T. Liu.** 1996. Effect of a 10-amino acid deletion on the oncogenic activity of latent membrane protein 1 of Epstein-Barr virus. *Oncogene* **12**, 2129-2135.
24. **Miller, W.E., R.H. Edwards, D.M. Walling, and N.R.-Traub.** 1994 Sequence variation in the Epstein-Barr virus latent membrane protein 1. *J. Gen. Virol.* **75**, 2729-2740.
25. **Mitchell, T. and B. Sugden.** 1995. Stimulation of NF-kappa B-mediated transcription by mutant derivatives of the latent membrane protein of Epstein-Barr virus. *J.*

Virol. **69**, 2968-2976.

26. **Moorthy, R.K. and D.A. Thorley-Lawson.** 1993. All three domains of the Epstein-Barr virus-encoded latent membrane protein LMP-1 are required for transformation of rat-1 fibroblasts. *J. Virol.* **67**, 1638-1646.
27. **Rickinson, A.B. and E. Kieff.** 1996. Epstein-Barr virus, p. 2397-2446. *In* Fields, B.N., D.M. Knipe, P.M. Howley, R.M. Chanock, J.L. Melnick, T.P. Monath, B. Roizman, and S. Straus (eds.), Fields Virology, 3rd ed. Raven Press, Philadelphia.
28. **Rosenthal, N.** 1988. Identification of regulatory elements of cloned genes. *Methods Enzymol.* **152**, 704-720.
29. **Rothenberger, S., E. Bachmann, C. Berger, C. McQuain, B. F. Odermatt, and H. Knecht.** 1997. Natural 30 base pair and 69 base pair deletion variants of the LMP1 oncogene do simulate NF-βB-mediated transcription. *Oncogene* **14**, 2123-2126.
30. **Rowe, M., M. Peng-Pilon, D.S. Huen, R. Hardy, D. Croom-Carter, E. Lundgren, and A.B. Rickinson.** 1994. Upregulation of bcl-2 by the Epstein-Barr virus latent membrane protein LMP1: a B-cell-specific response that is delayed relative to NF-kappa B activation and to induction of cell surface markers. *J. Virol.* **68**, 5602-5612.
31. **Sample, J., E.F. Kieff, and E.D. Kieff.** 1994. Epstein-Barr virus types 1 and 2 have nearly identical LMP-1 transforming genes. *J. Gen. Virol.* **75**, 2741-2746.
32. **Sample, J., L. Young, B. Martin, T. Chatman, E. Kieff, A. Rickinson, and E. Kieff.** 1990. Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B and EBNA-3c genes. *J. Virol.* **64**, 4084-4092.
33. **Sandvej, K. S.C. Peh, B. Andresen, and G. Pallesen.** 1994. Identification of potential hot-spots in the carboxy-terminal part of the Epstein-Barr virus (EBV) BNLF-1 gene in both malignant and benign EBV associated diseases: High frequency of a 30 bp deletion in Malaysian and Danish peripheral T-cell lymphomas. *Blood* **84**, 4053-4060.
34. **Sandvej, K., J.W. Gratama, M. Munch, X-. G. Zhou, R.L.H. Bolhuis, B.S. Andresen, N. Gregersen, and S. H-.Dutoit.** 1997. Sequence analysis of the Epstein-Barr virus (EBV) latent membrane protein-1 gene and promoter region: Identification of four variants among wild-type EBV isolates. *Blood* **90**, 323-330.
35. **Shim, Y.S., C.W. Kim, and W.K. Lee.** 1998 Sequence variation of EBNA2 of Epstein-Barr virus isolates from Korea. *Mol. Cells*, **8**, 226-232.
36. **Smir, B.N., R.J. Hauke, P.J. Bierman, T.G. Gross, F. d' Amore, J.R. Anderson, and T.C. Greiner.** 1996. Molecular epidemology of deletions and mutations of the latent membrane protein 1 oncogene of the Epstein-Barr virus in posttransplant lymphoproliferative disorders. *Laboratory Investigation* **75**, 575-588.
37. **Trividi, P., L.F. Hu, F. Chen, B. Christensson, M.G. Masucci, G. Klein, and G. Winberg.** 1994. Epstein-Barr virus (EBV)-encoded membrane protein LMP1 from a nasopharyngeal carcinoma in non-immunogenic in a murine model system, in contrast to a B cell-derived homologue. *Eur. J. Cancer.* **30A**, 84-88.
38. **Wang, D., D. Liebowitz, and E. Kieff.** 1985. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. *Cell* **43**, 831-840.
39. **Wang, F., C. Gregory, C. Sample, M. Rowe, d. Liebowitz, R. Murray, A. Ricknson, and E. Kieff.** 1990. Epstein-Barr virus latent membrane protein (LMP 1) and nuclear protein 2 and 3C are effectors of phenotypic changes in B lymphocytes: EBNA2 and LMP1 cooperatively induce CD23. *J. Virol.* **64**, 2309-2318.