

# Gene analysis of Epstein-Barr virus-associated lymphomas in Hu-PBL/SCID chimeras

Runliang Gan, Xiaoli Xie, Jie He, Xiaomin Liu, Li Hong, Yunlian Tang, Fang Liu, and Hailong Xie

Cancer Research Institute, University of South China, Hengyang City, Hunan 421001, China

---

## ABSTRACT

---

**Aims and background.** The mechanisms of Epstein-Barr virus (EBV)-associated tumor development are incompletely understood. The aim of this study was to investigate the gene expression of EBV-associated lymphomas in hu-PBL/SCID mice.

**Methods.** Human peripheral blood lymphocytes (hu-PBL) from EBV-seropositive donors were transplanted into severe combined immunodeficiency (SCID) mice. *In situ* hybridization was used to detect EBV-encoded small RNA-1 (EBER1) in tumor tissues. Mutation of *TP53* exons 5-8 in EBV-induced lymphomas was analyzed by PCR-SSCP. Immunohistochemical staining was used to examine EBV gene products and cellular oncoproteins.

**Results.** Twenty-one of 29 mice developed tumors. EBER1 was positive in the nuclei of almost all tumor cells. Immunohistochemistry showed positive staining of LMP1, EBNA2 and ZEBRA in a small number of tumor cells. Immunohistochemically detectable p53 protein expression was common (85.7%), but *TP53* gene mutations were identified in only four cases (19.1%) of EBV-associated lymphomas. Positivity rates of C-myc, Bcl-2 and Bax expression were 100%, 95.2%, and 90.5%, respectively, in the 21 cases of EBV-associated lymphomas.

**Conclusions.** Our preliminary findings suggest that EBV-associated lymphomas in hu-PBL/SCID chimeras show EBV infection, expression of oncogenic viral genes, and overexpression of cellular oncogenes. *TP53* gene mutations are rare but p53 protein is commonly expressed in EBV-associated lymphomas. Free full text available at [www.tumorionline.it](http://www.tumorionline.it)

---

## Introduction

Epstein-Barr virus (EBV), a member of the herpesvirus family, has been classified as a group 1 carcinogen associated with a variety of lymphoid and epithelial malignancies by the International Agency for Research on Cancer (IARC)<sup>1</sup>. Most EBV infections in infants and children are asymptomatic, while infections of adolescents or adults often result in infectious mononucleosis<sup>2</sup>. Evidence of EBV being an oncogenic virus is derived from its abilities to infect and transform normal human B-cells *in vitro*, resulting in the immortalization of these cells and leading to the continuous growth of lymphoblastoid cell lines. Moreover, EBV can transform human squamous epithelial cells *in vitro*, and clinically it is involved in the carcinogenesis of several human cancers such as nasopharyngeal carcinoma and various lymphomas<sup>3,4</sup>. The incidence of malignant lymphomas is significantly higher in patients who have congenital or acquired immunodeficiencies. About 50% of Hodgkin's and 20% of Burkitt's lymphomas in the United States contain EBV DNA<sup>5</sup>.

Since the development of human solid organ transplantation and the increase in AIDS cases in recent years, lymphoproliferative disorders in immunocompromised patients have become much more frequent. These disorders and their relationship with EBV have raised increasing interest<sup>6-8</sup>. Failure of the cellular immune response to control EBV-induced B-cell proliferation can result in severe disease<sup>9</sup>. In patients who have undergone

**Key words:** Epstein-Barr virus (EBV), induced lymphoma, oncogenes, *TP53*, hu-PBL/SCID chimera

**Acknowledgments:** Grant support was received from the National Natural Science Foundation of China (#30772116, #30801335), the Construct Program of the Key Discipline in Hunan Province, and the Opening Foundation of Key Lab of Hunan Province (09K075).

**Correspondence to:** Runliang Gan, MD, PhD, Cancer Research Institute, School of Medicine, University of South China, Chang Sheng Xi Avenue 28, Hengyang City, Hunan 421001, P.R. China.

Tel +86-734-8282923;  
fax +86-734-8281305;  
e-mail [gan998@yahoo.com](mailto:gan998@yahoo.com)

**Co-corresponding author:** Hailong Xie, MD, PhD, Cancer Research Institute, University of South China.

Tel +86-734-8281510;  
fax +86-734-8281305

Received August 25, 2009;  
accepted November 17, 2009.

organ transplants, the incidence of lymphoproliferative disorders varies from 0.5% after bone marrow transplantation to 10% after heart-lung transplantation. Most post-transplantation lymphomas are associated with EBV infection<sup>10</sup>.

The human peripheral blood lymphocyte-severe combined immunodeficiency (hu-PBL/SCID) chimera was constructed by implantation of hu-PBLs into SCID mice. It was used to generate EBV-associated lymphomas derived from human xenografts<sup>11-13</sup>. The findings obtained with this mouse model confirmed the significant role of immunodeficiency factors in the pathogenesis of lymphomas.

Since the mechanisms of EBV-associated tumor development are not fully understood, the purpose of the current study was to investigate the molecular characteristics of EBV-associated lymphomas in hu-PBL/SCID mice and to evaluate the impact of EBV latent gene expression in the tumor cells.

## Materials and methods

### *Construction of hu-PBL/SCID chimeras*

Fresh human peripheral blood (300 mL) was obtained from 12 healthy volunteers who gave their informed consent to the study. Prior EBV infection was confirmed by testing for the presence of serum antiviral capsid antigen (IgA/VCA+). Human peripheral blood lymphocytes were isolated by Ficoll-Hypaque gradient centrifugation, washed 4 times with RPMI-1640 medium, and then counted.

Twenty-nine SCID mice (homozygous C.B.-17 scid/scid) were purchased from the Laboratory Animal Center of the Chinese Science Academy (Beijing, China) and maintained in our facilities under special pathogen-free conditions. Groups of 4- to 6-week-old mice of both sexes were inoculated intraperitoneally with 8 to 10 × 10<sup>7</sup>/mL PBLs derived from 1 EBV-seropositive donor for each mouse. These mice are hereafter referred to as hu-PBL/SCID chimeras. The animals were observed every day for signs of illness; when they became sick, they were killed by exposure to excess ethyl ether and autopsied. Other SCID mice were kept alive to 135 days of follow-up and then killed and examined to discover whether or not tumors had developed in their bodies. The tumor specimens were divided in 2 parts: one was immediately frozen at -80 °C for DNA analysis and the other was fixed in 10% neutralized formalin, embedded in paraffin and sectioned at 4 μm for histopathological observation and immunohistochemical examination.

### *Immunohistochemistry*

Tumors were identified in 21 hu-PBL/SCID chimeras. Immunohistochemical stains were performed to assess:

(1) differentiated antigens of tumor cells, a panel of monoclonal antibodies against lymphocytes (LCA, CD45), B cells (L26, CD20) and T cells (CD45RO, CD3) (purchased from Maxim Biotech Inc., South San Francisco, CA, USA); (2) expression products of EBV genes (*LMPI*, *EBNA2*, *BZLF1*) (purchased from Dako Co., Kyoto, Japan); (3) expression analysis of p53, C-myc, Bcl-2 and Bax proteins in tumor cells using monoclonal antibodies (Maxim Biotech Inc.) according to the manufacturer's protocol. Briefly, endogenous peroxidase was quenched with 0.3% hydrogen peroxide. Sections were pre-treated for 15 minutes with antigen retrieval buffer. Before the application of the primary antibody, nonspecific binding was blocked with normal non-immune serum, and tissue slides were incubated with each diluted antibody at 4 °C overnight. Bound antibody was detected with biotin-conjugated secondary antibody followed by streptavidin-peroxidase and diaminobenzidine (DAB) color reagent. The slides were counterstained with hematoxylin. A case was considered positive if more than 5% of the malignant cells were stained with the antibody, although in practice most positive cases had 20% or more stained cells.

The p53, C-myc, Bcl-2 and Bax proteins were localized and quantified by microscopic evaluation of nuclear staining. The staining intensity was scored subjectively from + to +++, with (+) corresponding to less than 25% positive cells in the section, (++) corresponding to 25% to 50% positive cells, and (+++) corresponding to more than 50% positive cells.

### *PCR analysis*

DNA was extracted from tumor tissues as described in protocol (Ezra Blood DNA kit, purchased from OMEGA Bio-tek, USA). Alu PCR was carried out under the following conditions: 95 °C predenaturation for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 57 °C for 1 minute, and extension at 72 °C for 1 minute. Primers (sense 5'-CAC CTG TAA TCC CAG CAG TTT-3', anti-sense 5'-CGC GAT CTC GGC TCA CTG CA-3') were used to amplify a 221-bp sequence for human-specific *Alu*.

### *PCR-SSCP detection of TP53 gene mutations*

DNA extraction was performed according to the previous study<sup>11</sup>. The primer sequences of exons 5, 6, 7, and 8 of the *TP53* gene are listed below. Sense 5'-CTC TTC CTG CAG TAC TCC CCT GC-3', anti-sense 5'-GCC CCA GCT GCT CAC CAT CGC TA-3' was used to amplify a 221-bp sequence for exon 5. Sense 5'-GAT TGC TCT TAG GTC TGG CCC CTC-3', anti-sense 5'-GGC CAC TGA CCA CCA CCC TTA ACC-3' was used to amplify a 185-bp sequence for exon 6. Sense 5'-GTG TTG TCT CCT AGG TTG GCT CTG-3', anti-sense 5'-CAA GTG GCT CCT GAC CTG GAG TC-3' was used to amplify a 139-bp sequence for exon 7. Sense 5'-ACC TGA TTT CCT TAC TGC CTC

TGG C-3', anti-sense 5'-GTC CTG CTT GCT TAC CTC GCT TAG T-3' was used to amplify a 200-bp sequence for exon 8. The PCR was performed for 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 62 °C for 30 seconds, and extension at 72 °C for 40 seconds. The amplification products were analyzed by polyacrylamide gel electrophoresis.

SSCP analysis of PCR products was detected by the silver-staining method. Briefly, PCR products were mixed 1:1 with a sequencing stop solution. Samples were boiled at 95 °C for 5 minutes, cooled on ice and immediately loaded onto a 8% polyacrylamide-TBE gel containing 10% glycerol. Gels were run at 100 to 120 V for 1-2 hours at room temperature, washed with double-distilled water, and fixed in 10% ethanol for 10 minutes. They were then put into 1% HNO<sub>3</sub> for oxygenation and washed, dyed in 0.012 mol/L AgNO<sub>3</sub> for 20 minutes, and washed again. Next, the gels were soaked in 0.28 mol/L Na<sub>2</sub>CO<sub>3</sub> containing 0.019% methanol until bands appeared. The reaction was blocked immediately with 10% acetic acid because overexposure would darken the gel background. The presence of *TP53* gene mutation was indicated by the appearance of abnormal migrating bands in comparison with normal control.

#### *In situ hybridization*

The synthetic oligonucleotide EBER1 probe (sequence: 5'-CTC CTC CCT AGC AAA ACC CTC AGG ACG GCG-3')<sup>11</sup> was end-labeled with digoxigenin by tailing with terminal transferase. The labeling reaction was set up according to the manufacturer's protocol (Boehringer Mannheim, Ingelheim, Germany). *In situ* hybridization using EBER1 probe was carried out as follows. Briefly, the glass slides were pretreated with 2% APES. Four-micron tissue slices of the induced tumors were heated for 1 hour at 70 °C and deparaffinized in xylene, then digested with 0.5% mg/mL proteinase K for 10 minutes at 37 °C, and washed in water. The labeled probes were diluted to a concentration of 100 ng/mL in hybridization medium (25% deionized formamide, 4 × SSC (sodium citrate, sodium chloride), 50 mmol/L NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>, 1 mmol/L EDTA, 5 × Denhart's, 1 mg/mL ssDNA), and then spotted onto the tissue slices, which were covered with a coverslip. Both DNA probe and target RNA in tissue slices were simultaneously denatured at 70 °C for 8 minutes. Then the sections were hybridized at 37 °C for 4 hours or overnight. The non-specific or unbound probes were removed by 2 post-hybridization washes as follows: 2 × SSC, 0.1 × SSC, each for 10 minutes at room temperature. The slices were blocked with 2% normal goat serum at room temperature for 20 minutes, followed by incubation with anti-digoxigenin antibody at 37 °C for 30 minutes, then washed 3 times. NBT/NCIP was used as the chromogen. Counterstaining was done to enhance visualization with nuclear fast red.

## Results

### *Generation and pathological characteristics of the induced neoplasms in SCID mice*

Twenty-one of 29 hu-PBL/SCID mice developed tumors in their peritoneal cavities and/or mediastina. The time from hu-PBL transplantation to the death of mice with tumors varied from 31 days to 137 days, and the median survival time was 60 days. The tumors were solid, malacoid, irregular nodules (Figure 1A), non-encapsulated, adhering to surrounding organs and tissues. Their cut surfaces were gray-white or gray-red. A gray-yellow necrotic mass could often be seen in larger tumors. The neoplastic cells were polymorphic, diffuse large cells with differentiation of plasmacytoid or immunoblastic lymphocytes, containing large, round or slightly irregular nuclei and abundant cytoplasm (Figure 1B). Morphologically, all cases exhibited a diffuse infiltrative growth pattern. Tumor cells often infiltrated into the liver, kidney, pancreas (Figure 1C), lungs, and striated muscle of the posterior thoracic wall.

Immunohistochemical staining showed that all induced tumors were LCA (leukocyte common antigen) positive, B-cell marker (CD20) positive (Figure 1D), and T-cell marker (both CD3 and CD45RO) negative. Based on these morphological and immunohistochemical features, the tumors can be diagnosed as human B-cell lymphomas. Furthermore, *Alu*-PCR showed that all tumor tissues contained 221-bp *Alu* sequences (Figure 2). It confirmed that the induced tumors in hu-PBL/SCID chimeras were of human and not mouse origin.

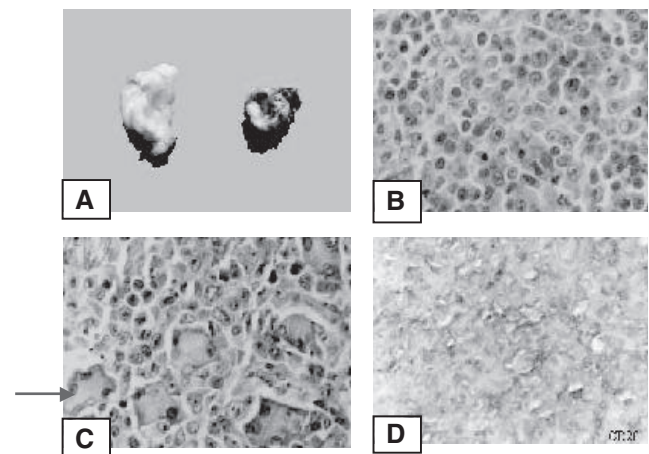


Figure 1 - Pathological features of the EBV-induced lymphomas. A) Fresh tumor tissues taken from hu-PBL/SCID chimeras were solid masses, gray-white and gray-red in color, 25 × 15 × 10 mm (left) and 10 × 8 × 5 mm (right) in size. B) The histopathological type of the induced tumors was diffuse large-cell lymphoma (HE stain, ×400). C) Diffuse large-cell lymphoma invading pancreatic tissue (arrow) of SCID mouse (HE stain, ×400). D) Immunohistochemistry revealed positive staining of B-cell markers (CD20, L26) on the membranes of tumor cells.

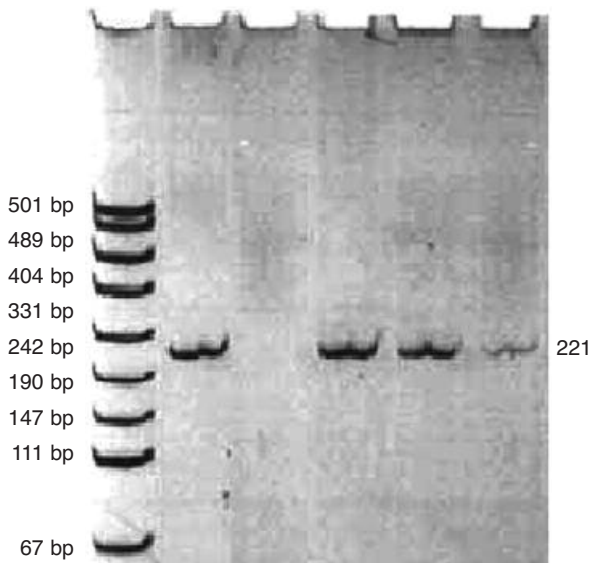


Figure 2 - PCR-amplified 221-bp products of human-specific Alu sequence of the EBV-induced tumors in SCID mice. M: DNA marker; P: human peripheral blood lymphocytes as positive control; N: murine liver tissue as negative control; T: tumor biopsy tissues.

#### Detection of EBV expression products

All of the 21 cases of tumors induced in hu-PBL/SCID mice were EBER1 positive. The *in situ* hybridization signal of the EBER1 probe was very intense with a diffuse pattern of staining confined to the nucleus (sparing the nucleus) in all cases. Notably, almost all morphologically malignant cells exhibited a positive signal with the EBER1 probe, while the normal host tissue adjacent to tumor cells was negative (Figure 3A). Additionally, immunohistochemistry showed that *BZLF1*-encoded ZEBRA protein was located in a small population of tumor cell nuclei (Figure 3B), EBNA2 was positive in the nuclei,

and LMP1 was expressed on the membranes of most tumor cells.

#### Mutation of *TP53* gene in EBV-associated lymphomas in hu-PBL/SCID chimeras

PCR amplification of *TP53* exons 5-8 was performed in all tumor tissues. The PCR products were verified by 8% polyacrylamide gel electrophoresis. Specific bands for 211-bp, 185-bp, 139-bp, and 200-bp products were identified and then analyzed by SSCP. Compared with controls, there were 5 mutations in 4 tumor cases (Figure 4) including 2 in exon 5, 2 in exon 6, and 1 in exon 7; one case showed 2 mutations (one in exon 5, another in exon 7). An abnormal migration pattern suggestive of the presence of mutation was identified in 4 of the 21 lymphomas; the mutation rate was 19.1% in all. To control for these, we analyzed each sample from the original hu-PBL donor as a control and made sure that the *TP53* gene alterations occurred during tumor development in the mouse and was not a germline polymorphism in the donor.

#### Detection of *p53*, *C-myc*, *Bcl-2*, and *Bax* proteins in EBV-associated lymphomas

The expression of 4 tumor-associated proteins was evaluated in 21 cases of EBV-associated lymphomas in hu-PBL/SCID chimeras by the immunohistochemical method. The positive rate of p53 protein expression (Figure 5A) in the 21 lymphomas was 85.7% (18/21 cases), although only 4 of the 18 cases with p53-immunoreactive tumors had abnormally migrating bands by PCR-SSCP analysis. The positive rates of *C-myc*, *Bcl-2*, and *Bax* (Figure 5B, 5C, 5D) expression in the 21 EBV-induced lymphomas were 100%, 95.2%, and 90.5%, respectively (Table 1). The rate of *Bcl-2* and *Bax* coexpression was 80.9%.

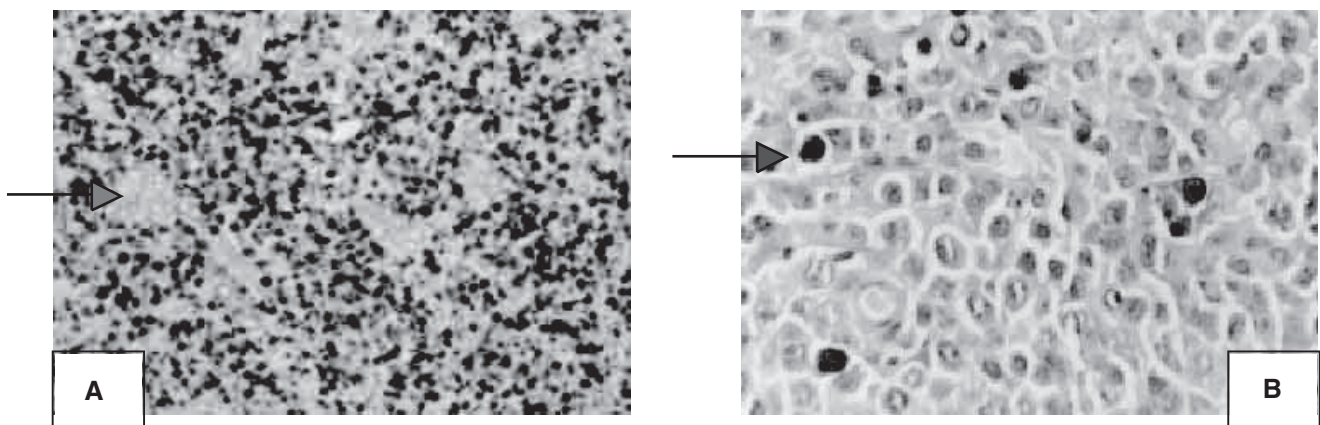


Figure 3 - Expression of EBV genome in the induced lymphoma of SCID mice. A) Positive signals of EBER1 were located in the nuclei of all tumor cells, whereas pancreatic epithelial cells (arrow) of SCID mouse were negative by *in situ* hybridization ( $\times 400$ ). B) Immunohistochemical staining of *BZLF1*-encoded protein was positive in a small number of tumor cells (arrow) and located in the nuclei of tumor cells (SP,  $\times 400$ ).

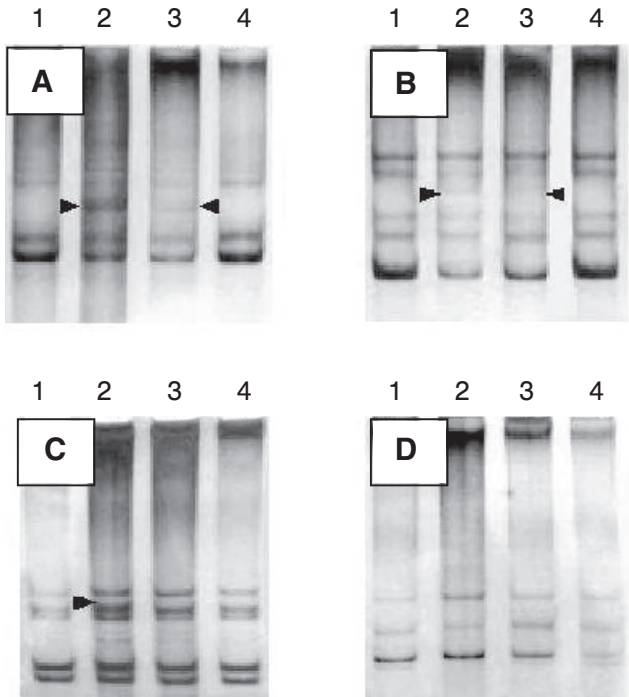


Figure 4 - PCR-SSCP analysis of p53 exons in EBV-associated tumors of SCID mice, where arrows indicate the appearance of abnormal migrating bands. Lane 1 is normal tissue and lane 2, 3, and 4 are tumor tissues. A) Lanes 2 and 3 showed abnormal migrating bands for exon 5. B) Lanes 2 and 3 showed abnormal migrating bands for exon 6. C) Lane 2 showed abnormal migrating bands for exon 7. D) No abnormal migrating band was seen for exon 8.

## Discussion

Histopathological examination of the induced tumors in hu-PBL/SCID mice demonstrated they were high-grade non-Hodgkin's lymphomas. We further proved that they were human B-cell lymphomas by detecting immune markers and *Alu* sequences. The *Alu* repeat sequence is unique in the human DNA genome. It is used to ascertain whether tumors are derived from humans. In this study, we used the *Alu*-PCR method to firmly establish that the EBV-induced tumors were derived entirely from human lymphocytes.

EBV is a lymphotropic herpesvirus. EBV-encoded small RNAs (EBERs) are the most abundant viral transcripts in latently EBV-infected cells. However, their roles in viral infection remain poorly understood. *In situ* hybridization of EBERs is the most sensitive and specific method to examine EBV. Our results showed that EBER1 was positive in the nuclei of almost all tumor cells but absent in murine cells around tumors. Recent studies have suggested a role for the EBERs in B-lymphocyte growth transformation<sup>14</sup>. EBERs confer resistance to Fas-mediated apoptosis by blocking PKR activity<sup>15</sup>. Now it appears that EBERs might play a key role in maintaining the malignant phenotype of Burkitt's lymphoma cells.

The EBERs induce clonability in soft agarose, tumorigenicity in mice, and resistance to apoptosis against various stimuli in Burkitt's lymphoma<sup>16</sup> and in human epithelial cells<sup>17</sup>. In addition, *BZLF1*-encoded protein (ZEBRA) is expressed in a small population of induced tumor cells. Other studies demonstrated that EBV's *BZLF1* gene product plays a central role in regulating the switch from latency to productive infection *in vitro*<sup>18,19</sup>, and furthermore, lytically infected cells may contribute to the growth of EBV-associated malignancies by enhancing angiogenesis in LCL tumors *in vivo*<sup>20</sup>. EBV-encoded latent membrane protein 1 (LMP1) has oncogenic potential and is expressed in many EBV-associated malignancies<sup>21</sup>. *In vitro*, LMP1 activates numerous signaling pathways including p38, c-Jun N terminal kinase (JNK), phosphatidylinositol 3 kinase (PI3K)/Akt, and NF-kappaB through interactions with tumor necrosis receptor-associated factors (TRAFs). These pathways are frequently activated in EBV-associated malignancies, although their activation cannot be definitively linked to LMP1 expression *in vivo*<sup>22</sup>. In the experiment described here, immunohistochemistry showed positive staining of LMP1, EBNA2 and ZEBRA in tumor cells. The results indicated that the pattern of EBV expression in EBV-induced B-cell lymphomas was latency type III. The molecular mechanism of the EBV-induced tumors was associated with EBV infection, expression of oncogenic viral genes, and abnormal expression of cellular oncogenes in human xenografts. EBV expression products might act directly on the infected cells or interfere with signal transduction of the infected cells, thereby inducing cell transformation. In our study, the infected cells proliferated and developed malignancy in an immunodeficient setting.

The tumor suppressor gene *TP53* is the most frequently mutated gene in human cancers and its mutations may result in the genesis and/or progression of tumors. Point mutations in *TP53* are associated with stabilization of the protein product and will lead to detectable overexpression of the protein. In the present study, however, we found a discrepancy between the frequency of p53 overexpression detected by immunohistochemistry and the frequency of gene mutations. This means that the mechanism of p53 accumulation in these tumors may be different from what has been described in other forms of cancer but similar to the reported mechanism in nasopharyngeal cancer or lymphomas<sup>23</sup>. Immunohistochemically detectable p53 protein expression is common in anaplastic large-cell lymphoma (65%), but *TP53* gene mutations were identified in only 3 of 36 cases (8%)<sup>24</sup>. The relatively low frequency of mutations in this study confirms previously reported findings that *TP53* gene mutation is not a frequent event in the tumorigenesis of lymphomas and the expression of p53 protein is independent of *TP53* mutations. Also nasopharyngeal cancer has a high frequency of p53 overexpression, but *TP53* mutations are rarely detect-

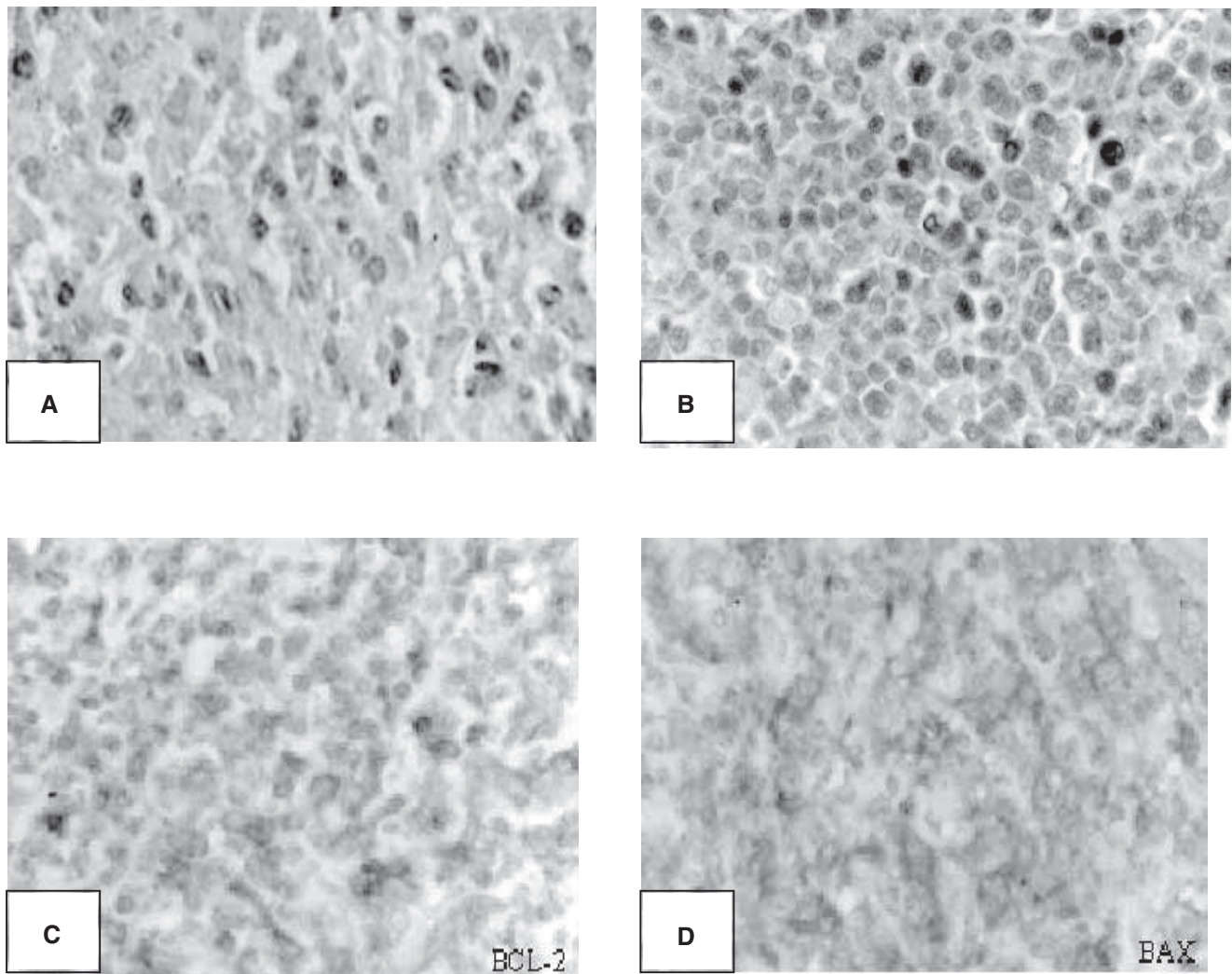


Figure 5 - Overexpression of cellular oncoproteins in the EBV-induced tumors. A) Immunoperoxidase staining with anti-p53 monoclonal antibody showing diffuse strong nuclear immunoreactivity for the p53 protein. B) C-myc was positive in the nuclei of neoplastic cells. C) Bcl-2 was positive in the cytoplasm and nuclear membrane of neoplastic cells. D) Bax was positive in the cytoplasm of neoplastic cells by immunohistochemical staining. (SP,  $\times 400$ ).

**Table 1 - Expression of p53, C-myc, Bcl-2, and Bax proteins in EBV-associated lymphomas**

Protein expression	Negative cases	Positive cases				Positivity rate (%)
		+	++	+++	Total	
p53	3	1	4	13	18	85.7
C-myc	0	2	7	12	21	100
Bcl-2	1	1	5	14	20	95.2
Bax	2	2	7	10	19	90.5

Note: Twenty-one cases of tumors have been identified in hu-PBL/SCID mice by means of pathological examination (N = 21).

ed<sup>25</sup>. Even though the reason for the discrepancy between *TP53* mutation and p53 overexpression remains unclear, it is suggested that p53 overexpression may be involved in the process of malignant transformation in these induced lymphomas.

It is known that Burkitt's lymphoma is very closely associated with EBV and *C-myc* is activated in almost all Burkitt's lymphomas<sup>26</sup>. These 2 facts indicate that EBV may be involved in *C-myc* activation. Our results showed that C-myc protein was expressed in all EBV-induced lymphomas, and further suggest that EBV infection may activate *C-myc* and then promote the development of lymphomas. LMP1 was expressed on the membrane of tumor cells in all 21 positive cases with Bcl-2. EBV LMP1 can enhance Bcl-2 expression to block cell apoptosis. Bcl-2, an antiapoptotic protein, is upregulated by LMP1 and also overexpressed in nasopharyngeal cancer<sup>27</sup>. When *Bcl-2* antisense oligodeoxynucleotide was transferred into malignant lymphoid cells, the expression of Bcl-2 protein decreased, cell proliferation was inhibited, and the number of apoptotic cells increased<sup>28</sup>. Moreover, Bax homodimerizes or het-

erodimerizes with Bcl-2 *in vivo*. The ratio of Bcl-2 to Bax affects the rate of apoptosis: the former promotes and the latter inhibits apoptosis<sup>29</sup>. We used immunostaining to detect the expression of Bcl-2 and Bax in EBV-induced tumors. The positive rates were 95.2% and 90.5%, respectively, and the coexpression rate of Bcl-2 and Bax was 87.5%. These results suggest that EBV may upregulate Bcl-2 expression and make it bind to Bax competitively, thereby protecting cells from apoptosis and promoting tumorigenesis.

Epstein-Barr virus-associated lymphoproliferative disease (EBV-LPD) is a rare but serious complication in recipients of organ and stem cell transplants. In the present EBV-associated lymphoma model, the EBV-associated lymphomas in hu-PBL/SCID chimeras showed EBV infection, expression of oncogenic viral genes, and overexpression of cellular oncogenes. *TP53* gene mutation was rare but p53 protein was commonly expressed in EBV-associated lymphomas. Except for gene mutation, other pathways may be involved in the accumulation of p53 protein in EBV-induced tumors. It is important for us to establish an EBV-associated lymphoma model in an immunodeficient setting and to further investigate the molecular mechanism of EBV-associated lymphoma development.

## References

- Boccardo E, Villa LL: Viral origins of human cancer. *Curr Med Chem*, 14: 2526-2539, 2007.
- Balfour HH Jr, Holman CJ, Hokanson KM, Lelonek MM, Giesbrecht JE, White DR, Schmeling DO, Webb CH, Cavert W, Wang DH, Brundage RC: A prospective clinical study of Epstein-Barr virus and host interactions during acute infectious mononucleosis. *J Infect Dis*, 192: 1505-1512, 2005.
- Li HM, Man C, Jin Y, Deng W, Yip YL, Feng HC, Cheung YC, Lo KW, Meltzer PS, Wu ZG, Kwong YL, Yuen AP, Tsao SW: Molecular and cytogenetic changes involved in the immortalization of nasopharyngeal epithelial cells by telomerase. *Int J Cancer*, 119: 1567-1576, 2006.
- De Sanjosé S, Bosch R, Schouten T, Verkuijlen S, Nieters A, Foretova L, Maynadié M, Cocco PL, Staines A, Becker N, Brennan P, Benavente Y, Boffetta P, Meijer CJ, Middeldorp JM: Epstein-Barr virus infection and risk of lymphoma: immunoblot analysis of antibody responses against EBV-related proteins in a large series of lymphoma subjects and matched controls. *Int J Cancer*, 121: 1806-1812, 2007.
- Cohen JI: Benign and malignant Epstein-Barr virus-associated B-cell lymphoproliferative diseases. *Semin Hematol*, 40: 116-123, 2003.
- Frey NV, Tsai DE: The management of posttransplant lymphoproliferative disorder. *Med Oncol*, 24: 125-136, 2007.
- Carbone A, Ghoghini A: AIDS-related lymphomas: from pathogenesis to pathology. *Br J Haematol*, 130: 662-670, 2005.
- Meijer E, Cornelissen JJ: Epstein-Barr virus-associated lymphoproliferative disease after allogeneic haematopoietic stem cell transplantation: molecular monitoring and early treatment of high-risk patients. *Curr Opin Hematol*, 15: 576-585, 2008.
- Sebelin-Wulf K, Nguyen TD, Oertel S, Papp-Vary M, Trappe RU, Schulzki A, Pezzutto A, Riess H, Subklewe M: Quantitative analysis of EBV-specific CD4/CD8 T cell numbers, absolute CD4/CD8 T cell numbers and EBV load in solid organ transplant recipients with PLTD. *Transpl Immunol*, 17: 203-210, 2007.
- Annels NE, Kalpoe JS, Bredius RG, Claas EC, Kroes AC, Hislop AD, van Baarle D, Egeler RM, van Tol MJ, Lankester AC: Management of Epstein-Barr virus (EBV) reactivation after allogeneic stem cell transplantation by simultaneous analysis of EBV DNA load and EBV-specific T cell reconstitution. *Clin Infect Dis*, 42: 1743-1748, 2006.
- Gan R, Yin Z, Liu T, Wang L, Tang Y, Song Y: Cyclosporine A effectively inhibits graft-versus-host disease during development of Epstein-Barr virus-infected human B cell lymphoma in SCID mouse. *Cancer Sci*, 94: 796-801, 2003.
- Islas-Ohlmayer M, Padgett-Thomas A, Domiati-Saad R, Melkus MW, Cravens PD, Martin Mdel P, Netto G, Garcia JV: Experimental infection of NOD/SCID mice reconstituted with human CD34+ cells with Epstein-Barr virus. *J Virol*, 78: 13891-13900, 2004.
- Lim WH, Kireta S, Russ GR, Coates PT: Human plasmacytoid dendritic cells regulate immune responses to Epstein-Barr virus (EBV) infection and delay EBV-related mortality in humanized NOD-SCID mice. *Blood*, 109: 1043-1050, 2007.
- Yajima M, Kanda T, Takada K: Critical role of Epstein-Barr virus (EBV)-encoded RNA in efficient EBV-induced B-lymphocyte growth transformation. *J Virol*, 79: 4298-4307, 2005.
- Clemens MJ: Epstein-Barr virus: inhibition of apoptosis as a mechanism of cell transformation. *Int J Biochem Cell Biol*, 38: 164-169, 2006.
- Nanbo A, Takada K: The role of Epstein-Barr virus-encoded small RNAs (EBERs) in oncogenesis. *Rev Med Virol*, 12: 321-326, 2002.
- Nanbo A, Yoshiyama H, Takada K: Epstein-Barr virus-encoded poly (A)-RNA confers resistance to apoptosis mediated through Fas by blocking the PKR pathway in human epithelial intestine 407 cells. *J Virol*, 79: 12280-12285, 2005.
- Petosa C, Morand P, Baudin F, Moulin M, Artero JB, Muller CW: Structural basis of lytic cycle activation by the Epstein-Barr virus ZEBRA protein. *Mol Cell*, 21: 565-572, 2006.
- Ladell K, Dorner M, Zauner L, Berger C, Zucol F, Bernasconi M, Niggli FK, Speck RF, Nadal D: Immune activation suppresses initiation of lytic Epstein-Barr virus infection. *Cell Microbiol*, 9: 2055-2069, 2007.
- Hong GK, Kumar P, Wang L, Damania B, Gulley ML, Delecluse HJ, Polverini PJ, Kenney SC: Epstein-Barr virus lytic infection is required for efficient production of the angiogenesis factor vascular endothelial growth factor in lymphoblastoid cell lines. *J Virol*, 79: 13984-13992, 2005.
- Kobayashi H, Nagato T, Takahara M, Sato K, Kimura S, Aoki N, Azumi M, Tateno M, Harabuchi Y, Celis E: Induction of EBV-latent membrane protein 1-specific MHC class II-restricted T-cell responses against natural killer lymphoma cells. *Cancer Res*, 68: 901-908, 2008.
- Thornburg NJ, Kulwicht W, Edwards RH, Shair KH, Bendt KM, Raab-Traub N: LMP1 signaling and activation of NF-kappaB in LMP1 transgenic mice. *Oncogene*, 25: 288-297, 2006.
- Shaminie J, Peh SC, Tan J: P53 alterations in sequential biopsies of Asian follicular lymphoma: a study of immunohistochemical staining pattern and gene mutations by PCR-SSCP in paraffin-embedded tissues. *Pathology*, 37: 39-44, 2005.
- Rassidakis GZ, Thomaidis A, Wang S, Jiang Y, Fourtouna A, Lai R, Medeiros LJ: P53 gene mutations are uncommon but p53 is commonly expressed in anaplastic large-cell lymphoma. *Leukemia*, 19: 1663-1669, 2005.

25. Sun Y, Yi H, Zhang PF, Li MY, Li C, Li F, Peng F, Feng XP, Yang YX, Yang F, Xiao ZQ, Chen ZC: Identification of differential proteins in nasopharyngeal carcinoma cells with p53 silence by proteome analysis. *FEBS Lett*, 581: 131-139, 2007.
26. Brady G, MacArthur GJ, Farrell PJ: Epstein-Barr virus and Burkitt lymphoma. *J Clin Pathol*, 60: 1397-1402, 2007.
27. Burgos JS: Involvement of the Epstein-Barr virus in the nasopharyngeal carcinoma pathogenesis. *Med Oncol*, 22: 113-121, 2005.
28. Chanan-Khan A: Bcl-2 antisense therapy in B-cell malignancies. *Blood Rev*, 19: 213-221, 2005.
29. Del Principe MI, Del Poeta G, Venditti A, Buccisano F, Maurillo L, Mazzone C, Bruno A, Neri B, Irno Consalvo M, Lo Coco F, Amadori S: Apoptosis and immaturity in acute myeloid leukemia. *Hematology*, 10: 25-34, 2005.