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이학석사 학위논문

**Establishment and Characterization of  
P -  
DEpstein-Barr Virus Cell Lines**

암환자로부터 유래된  
EBV 양성 B 림프구 세포주의 수립과 특성분석 연구

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# **Abstract**

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The international agency for research on cancer estimates that one in five cancer cases worldwide are caused by infection, with most caused by viruses. Viruses have been central to modern cancer research and provide profound insights into both infectious and non-infectious cancer causes. Many studies have been conducted on this, and the link between virus infection and cancer development is increasing (1, 2).

Epstein-Barr virus (EBV), one of the typical virus that causes cancer, is a  $\gamma$ -herpes viruses. EBV infection of resting B lymphocytes *in vitro* give rise to spontaneous outgrowth of EBV-transformed cell lines, referred to as lymphoblastoid cell lines (LCLs) in previous studies (2, 3). But, in this study, the cell lines established by the proliferation of EBV-infected lymphocytes in tissue during cell culture for establishment of tumor cell lines are referred LCLs.

These LCLs are easy to handle and to maintain so that they are suitable material for biological research (4). Although there are many studies relating LCLs with cancer therapy (5, 6, 7), there has not yet been a study to confirm the presence of anticancer

effect in the same patient by using LCLs itself.

Therefore, we performed type analysis and characterization of LCLs which were established by *in vitro* proliferation of EBV-infected lymphocytes in tissues during cell culture for establishment of tumor cell lines and we tried to show anticancer effect by approach of new immunotherapy for cancer by these LCLs having EBV specificity.

All LCLs were found to have different origins, EBV was predominantly infected with lymphocytes, and EBNA 1, EBNA 3A, EBNA 3C, BARF 1 and LMP 1 genes were expressed by EBV infection. Using a specific cell surface marker, we could prove that most of the LCLs correspond to B cells. The proliferation assay of LCL having the characteristics of EBV showed that it was sensitive to ganciclovir, and in order to confirm whether it had an anticancer effect in fact, co-culture with cancer cell was performed *in vitro* and identified that cancer growth tended to be suppressed.

In this study revealed that characterization of EBV positive B-lymphoblastoid cell lines naturally derived from cancer patients by wright staining, PCR, RT-PCR, western blot, Flow cytometry and cell proliferation assays, and identified that LCLs induce apoptosis of cancer cell line. I think that this steady study of LCLs could act as a biomaterial that can be used to treat cancer and disease related to EBV in the future.

**Key words :** Epstein-Barr virus, B-lymphoblastoid cell line, latent gene, co-culture, apoptosis

**Student number :** 2015-22061

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## Introduction

(8)

in previous studies But, in this study, the cell lines established by the proliferation of EBV-infected lymphocytes in tissue during cell culture for establishment of tumor cell lines are referred LCLs.

(10)

(12) Also t (13) These viral latent genes play an important role in the maintenance replication and transcription of the EBV genome as well as the immortal of LCLs. Also, based on DNA sequence divergence in the EBNA 2 and EBNA 3 regions, the EBVs have been classified into type 1 (EBV-1) and type 2 (EBV-2) and show distinct biological differences such as reactivation capability and transforming ability (14). (11) and there is a difference in distribution by type according to region. Type 1 is distributed widely in East Asia including Europe, America and China, type 2 is mainly distributed in Africa and Papua New Guinea. On the other hand, according to recent research results, compared with the previous studies, the type 2 is gradually increasing from normal people in addition to the epidemic area, and intertypic infections are also increasing in normal people (15).

Studies on viruses (especially EBV), which cause 10-15% of cancer worldwide, are ongoing, and the link between virus infection and cancer development is on the rise

(1,2). In addition, many studies related to LCLs having the characteristics of such virus have been carried out because LCLs is easy to handle and maintain and is used as a material suitable for biological research (4, 5, 6, 7). But, there has not yet been a study to confirm the presence of anticancer effect in the same patient by using LCLs itself. So, I conducted experiments to use LCLs identified the anticancer effect.

In this study, I demonstrated that LCLs is EBV positive B-lymphoblastoid cell line through the characterization. Also, I confirmed the induction of apoptosis in cancer cell due to secreted substances of LCLs with EBV characteristics. I think that this may provide additional guidelines for the treatment of cancers and diseases associated with EBV.

## **Materials and methods**

### **Establishment and maintenance of Epstein-Barr virus (EBV) cell lines**

EBV - grown , Thermo scientific., MA, USA/ L () B95-8, Jiyoye and SNU-1750 cell lines were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea).

## Detection of mycoplasma contamination

Genomic DNA was isolated from cell pellets using QIAamp DNA mini kit (Qiagen, Hilden, Germany) and all of gDNA of LCLs, positive control that containing in kit, and negative control were amplified using thermal cycler (PCR system 9700, Applied Biosystems; CA, USA) with following conditions: initial denaturation for 5 min at 94°C, cycling at 94°C (30 sec), 58°C (20 sec), and 72°C (30 sec) for 35 cycles, with final elongation for 7 min at 72°C. To confirmed specificity of contamination, PCR products were analyzed with gel electrophoresis using 2% agarose gel.

30 Epstein-Barr Virus cell lines (Table 2) were obtained from Korean Cell Line Bank. All cell lines were maintained in RPMI1640 medium (GE Healthcare Life Sciences) supplemented with 20% fetal bovine serum, penicillin (10,000U/ml), and streptomycin (10,000ug/ml). cell lines were grown at 37°C in a humidified atmosphere of air containing 5% CO<sub>2</sub> (2).

For DNA fingerprinting analysis to authenticate each cell lines, t 9700

## **Wright staining**

LCLs were harvested and washed three times with Phosphate Buffered Saline (PBS). The cell pellet was re-suspended in 100  $\mu$ L of medium and smeared on the slide glass. After air dry, dip into the wright solution (Sigma., MO, USA) for 3 min and then dip into distilled water. Subsequently, slide glasses of each cell lines were observed by microscopy.

## **Growth properties and morphology in vitro**

*Nucleic acid isolation and cDNA synthesis*Qiagen). For the synthesis of the cDNA, 1  $\mu$ g of total RNA was mixed with a random primer and Diethyl pyrocarbonate (DEPC) in a total volume of 12  $\mu$ L. The

PCR was applied for the detection of the human pathogenic viruses EBV and other  $\mu$ L  $\mu$ L mixture and us and the primer sequences that were used in this study were

listed on Table 3. PCR conditions for the reaction were 35 cycles were run template specific annealing temperature for nwere fractionated on a 1.5% agarose gel.

### *DNA profiles*

### *DNA fingerprinting analysis*

### *Mycoplasma test*

PCR-based techniques were progressed for detecting mycoplasma and used e-Myco™ plus mycoplasma PCR detection kit (Intron).

PCR conditions were

Initial denaturation 94°C at 1min, denaturation 94°C at 30sec, annealing 58°C at 20sec, extension 72°C at 1min, final extension 72°C at 5min, final hold 4°C.

### **Western blotting analysis**

LCL using containing protease-, phosphatase inhibitors ) To block the membrane, it was incubated in 2% skim milk and 0.5% Tween 20-TBS buffer containing 1mM of MgCl<sub>2</sub> for 2 hr at room temperature. Pagainst 1 Santa Cruz Biotechnology, CA, USA (1:200), CD45 (Santa Cruz) (1:100), CD21 (Santa Cruz) (1:100) and  $\beta$ -actin (Applied Biological Materials Inc., BC, Canada) (1:200) were incubated overnight at

4°C. Peroxidase conjugated mouse or rabbit IgG antibody (Jackson Immunoresearch, PA, USA) (1:5000) were incubated 1 hr at RT. Chemiluminescent working solution, Sensido ECL substrate (HRP) (Recenttec Inc., Taipei, Taiwan) was decanted to the membrane. The membrane was exposed to Fuji RX film (Fujifilm, Tokyo, Japan) for 1–5 min.

Each proteins were extracted by RIPA solution ( ) and were run on Mini-PROTEAN® TGX™ Gels (BioRad) as described above and subsequently transferred to nitrocellulose membranes using a semidry blot format (Trans-Blot Turbo Transfer System; BioRad). Following protein transfer, the membranes were blocked with blocking buffer (5% nonfat powdered milk buffered in Tris-HCl, pH 8.0, 150Mm NaCl, and 0.05% Tween 20 (TBST)). The individual proteins were diluted in 2% skim milk at dilutions varying from 1:500 to 1:2000 and incubated with the membranes for at least 1h at room temperature or overnight at 4°C. Following incubation with the primary antibody, the membranes were washed three times for 10minutes each with TBST. After washing, the secondary antibody (goat anti-mouse IgG) was diluted in 2% skim milk at 1 : 5,000 and incubated with the membrane for at least 1h at room temperature. The membranes were washed third with

TBST(3).

### *PCR assays and Reverse Transcription-PCR assays*

Total RNA was isolated using easy-BLUE kits (Intron Biotechnology, Korea) from cultured, and washed cell pellets. Briefly, the protocol involves adding 1ml of easy-BLUE to collected cells, vortexing vigorously, and then adding chloroform. After centrifuging the aqueous layer was transfer to a new tube. Isopropanol was added to precipitate RNA, and RNA pellets obtained were washed with 75% ethanol. Pellets were then dissolved in DEPC treated distilled water at 65°C for 20min, then stored at -70°C(4).

#### **For cDNA synthesis, 1ug of total RNA**

To identify EBV infected cell lines, PCR was performed using EBV, EBNA3A, EBNA3C primers and RT-PCR was performed using EBNA1, BARF1, LMP1 primers.

PCR general conditions were

Pre-denaturation 94°C at 5min, denaturation 94°C at 30sec, annealing at 30sec, extension 72°C at 1min, final extension 72°C at 7min, final

hold 4°C.

Each primer conditions were noted in table1 and using i-Taq™ DNA polymerase (Intron).

Surface antigens of LCLs were stained with antibodies (Abs) against, CD68 (FITC) (Santa Cruz),, , CD27 (FITC), IgD (BV510) ( (Abcam., CB, UK) (Cell signaling, Beverly, MA) (Genetex, CA, USA) for 1 hr at RT To determined dead cell population, cells were stained with pPI, μL or (BD Biosciences). Stained cells were To stain RT then anti- antibody r DFACS Canto (BD Biosciences).

**Cell proliferation assay** NADH-dehydrogenase in the live cell using Ez-Cytox(× inhrThen, Cat no.S1878, Selleckchem, USA)rEz-Cytox by a MULTISKAN FC Microplate Photometer (Thermo-Fisher Scientific, MA, USA)

$1 \times 10^5$  SNU-2550 cancer cells were seeded in 60-mm culture dishes. Seeded cells co-cultured with SNU-2550BL ( $2 \times 10^5$  cells/mL) (SPL life sciences, and were incubated for 8 days without media change (SNU-2550 cancer cells ( $2 \times 10^5$  cells/mL) co-cultured with SNU-1460BL ( $4 \times 10^5$  cells/mL) and HT-29 cancer cells ( $0.5 \times 10^5$  cells/mL) co-cultured with SNU-2550BL ( $1.5 \times 10^5$  cells/mL).

This process was repeated three times for each cell lines.

### **Apoptosis assay**

Cells were seeded in 60-mm culture dishes. After 24 hr, seeded cells co-cultured with LCLs using filter dishes and then after 5 hr, cells floating in the medium were collected. The adherent cells were detached with 2% trypsin. The cells were centrifuged for 3 min at 1,000 rpm and the supernatant was removed and cells were washed with PBS. After that cells were re-suspended with  $1 \times$  Annexin V binding buffer (Bioss, OR, USA) and add Annexin V antibody conjugated with Alexa 488 (Life technologies, OR, USA). Incubated the cells at room temperature for 15 min in dark and add PI ( $100 \mu\text{g/mL}$ )  $1 \mu\text{L}$ . The cells were then introduced to a flow cytometry to determine the proportions of the cell apoptosis.

### **Statistical analysis**

For statistical analysis, GraphPad Prism 5.03 (GraphPad Software Inc., San Diego, CA, USA) was used. In the Annexin V staining assay, the ratio of cells at each

apoptotic stage was expressed as the mean  $\pm$  standard deviation (SD). Statistical differences were tested with Student's *t*-test and a value of  $P < 0.05$  was considered statistically significant.

## Results

**Table 1. Origin of the Epstein-Barr virus (EBV) infected lymphoblastoid cell lines**

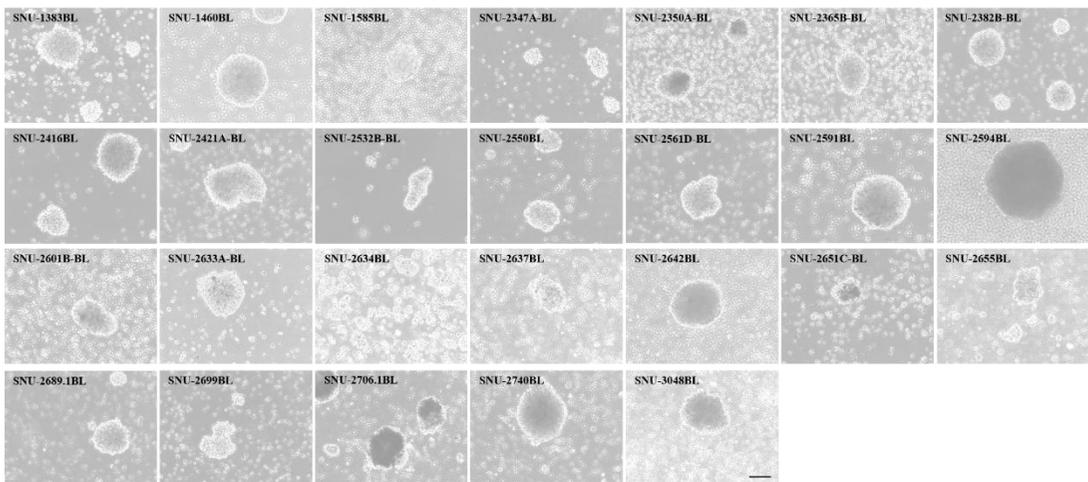
No.	Cell line	Date of initiation	Sex/ Age	Original diagnosis	Culture site
1	SNU-1383BL	14 June 1995	F/38	Rectal cancer	
2	SNU-1460BL	02 October 1996	M/25	Colorectal cancer	
3	SNU-1585BL	20 April 1999	M/47	Lung cancer	
4	SNU-2347A-BL	16 July 2007	M/50	Colorectal cancer	
5	SNU-2350A-BL	23 July 2007	M/76	Rectal cancer	
6	SNU-2365B-BL	13 September 2007	M/50	Colorectal cancer	liver metastasis
7	SNU-2382B-BL	10 January 2008	M/71	Colorectal cancer	serosal side
8	SNU-2416BL	18 August 2008	M/82	Colorectal cancer	
9	SNU-2421A-BL	10 October 2008	M/61	Small bowel cancer	
10	SNU-2532B-BL	27 October 2010	F/32	Breast cancer	
11	SNU-2550BL	21 February 2011	F/35	Lung cancer	
12	SNU-2561D-BL	13 April 2011	F/43	Colorectal cancer	
13	SNU-2591BL	18 November 2011	M/66	Lung cancer	
14	SNU-2594BL	30 November 2011	M/76	Lung cancer	
15	SNU-2601B-BL	27 January 2012	F/55	Liver metastasis	
16	SNU-2633A-BL	26 July 2012	M/69	Colorectal cancer	
17	SNU-2634BL	27 July 2012	F/51	Lung cancer	
18	SNU-2637BL	06 September 2012	M/42	Lung cancer	
19	SNU-2642BL	25 September 2012		Gastric cancer	
20	SNU-2651C-BL	07 November 2012		Liver metastasis	
21	SNU-2655BL	23 November 2012		Liver cancer	
22	SNU-2689.1BL	18 April 2013	F/74	Lung cancer	
23	SNU-2699BL	07 May 2013		Lung cancer	
24	SNU-2706.1BL	28 May 2013		Lung cancer	
25	SNU-2740BL	19 August 2013	M/56	Lung cancer	
26	SNU-3048BL	06 January 2015		Gastric cancer	

All twenty-six LCLs used in the experiment derived from cancer cell lines such as

colon cancer, lung cancer, breast cancer, liver cancer and stomach cancer.

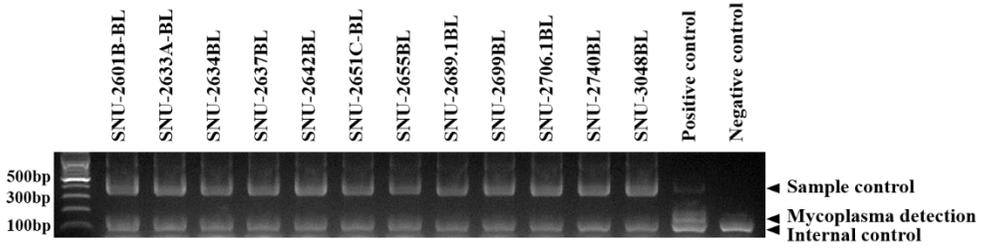
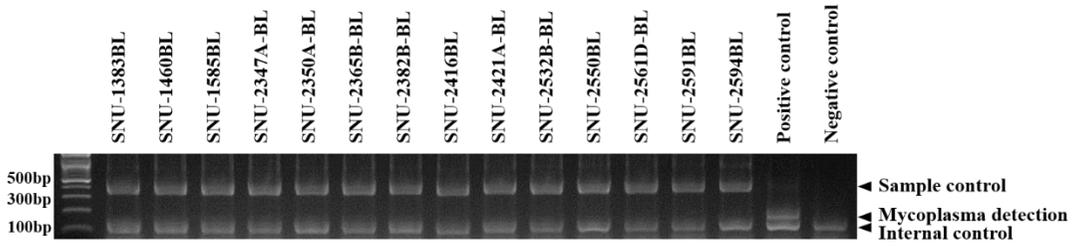
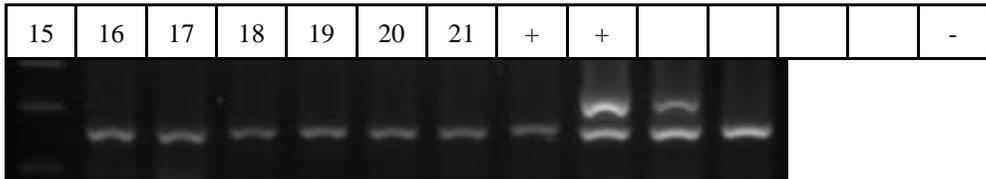
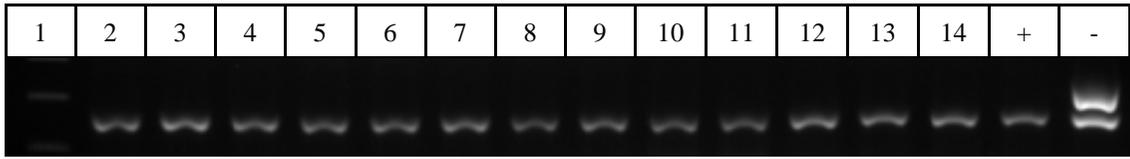
### **Morphological character of established Epstein-Barr virus (EBV) positive B-lymphoblastoid cell lines *in vitro***

twenty-six Twenty-four LCLs SNU-2347A-BL and SNU-2634BL



**Figure 1. Phase-contrast microscopy of Epstein-Barr virus (EBV) infected B-lymphoblastoid cell lines.** The twenty-six of suspended EBV cell lines were agglomerate together to form larger clusters and morphology of spike-like form. Scale bar is 100  $\mu\text{m}$ .

## Detection of mycoplasma contamination



**Figure 2. Detection of mycoplasma in Epstein-Barr virus (EBV) infected B-lymphoblastoid cell lines.** All twenty-six LCLs were free of contamination by mycoplasma.

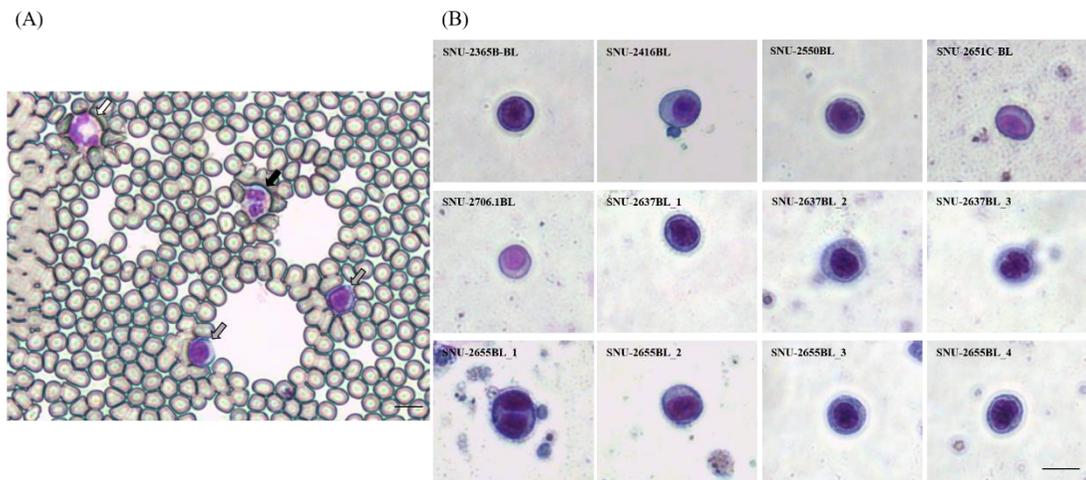
**Table 2. of Epstein-Barr virus (EBV) cell lines**

No	Cell line	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	Vwa	TPOX	D18S51	Amelogenin	D5S818	FGA
1	SNU-1383BL	15	30	10,11	12	14,18	6	11	9,11	17,18	13,14,2	17,18	11	13,14	X	10,12	19
2	SNU-1460BL	13	30,2,31	8,11	12	15,17	6,7	9,12	10	23,24	13,14,2	14,18	8,11	13,14	X.Y	11	21,23
3	SNU-1585BL	11,13	30	10,11	10,12	16	7	8	10,12	18,23	14,14,2	18,20	8,9	14,19	X.Y	11,12	23,24
4	SNU-2347A-BL	15,16	30,31	8,10	10	15,17	9	11	12	23,24	13,14	17,18	8,11	15,19	X.Y	9,10	22,25
5	SNU-2350A-BL	13,15	30,31	12,13	10,12	15,16	7,9	9,12	10,13	19,20	13,2,15	14,16	8,12	15,18	X.Y	11	20,22
6	SNU-2365B-BL	13,14	30,31,2	8,12	9,11	15,16	6,9	10,12	10,14	19,25	13,15,2	16,17	8,11	13,21	X.Y	9,14	20
7	SNU-2382B-BL	12,13	29,32	8,11	10,11	16	9	8,10	9,13	18,19	14,15,2	14,16	11	13,18	X.Y	8,11	24
8	SNU-2416BL	14,15	29	8,11	11	15,17	9,3,10	8,11	9,11	17,18	14,2,15,2	16	8,9	15	X.Y	10,12	21,23
9	SNU-2421A-BL	13,16	31,32,2	11,12	11,12	16,17	7,9	11	12,13	20,22	13,2,14	17,19	8,12	13,17	X.Y	10,14	21,23
10	SNU-2532B-BL	10,13	29,32	10,12	9,11	15,16	6,9	8	10,11	20,22	14,2	18,20	10,11	13,21	X	10,12	20,26
11	SNU-2550BL	14,15	29,30	8,12	10	15	9	8,11	9,13	23,24	12,15,2	14,18	11	15,17	X	11,12	19
12	SNU-2561D-BL	12,15	30,32,2	8,10	10,12	15,16	9	9,10	11,12	19,23	12,15,2	18	11	14	X	9,11	23,24
13	SNU-2591BL	14,15	28,30	9,11	12	15,17	7,9	10,11	10,11	23	14,2	14,17	8,11	13,18	X.Y	11,12	22,2,23
14	SNU-2594BL	11,15	30,32,2	8,12	10,11	15,17	7,8	8,9	10,12	21,25	14,14,2	16,17	11	14,16	X.Y	10,12	23
15	SNU-2601B-BL	12,14	30,31,2	10,12	11,12	16	7,8	12	10,11	23	14,15,2	17,18	8	17,19	X.Y	12,13	20,24
16	SNU-2633A-BL	14,15	30,33,2	9,11	11,14	15	7,9	8	9,13	22,25	13,2,14	17,18	8,9	13,20	X.Y	10,13	23,25
17	SNU-2634BL	12,13	31,33,2	8,14	11	15	7,8	8,10	9,13	23,25	14,15,2	17,19	8,11	15,18	X	10,11	23,25
18	SNU-2637BL	12,13	30,31,2	8,9	9,13	16,17	9	9,11	9	18,25	14,2,15,2	18,19	8	13,21	X.Y	11	22,25
19	SNU-2642BL	12,15	31,2,32,2	9,11	12	16	6,7	8,14	10,11	18,24	15,2	17	11	12,13	X	9,12	24
20	SNU-2651C-BL	12,13	30,31	9,11	9,11	15	7	8,13	8,9	18,21	14,2,15,2	14,17	10,11	15,17	X.Y	8,11	18,28
21	SNU-2655BL	13,14	30,32,2	8,10	10,12	15,16	7,9	11,14	12,13	18,24	13,14	14,16	8,11	14,16	X.Y	10,12	19,24
22	SNU-2689.1BL	11,14	30	11	12,14	15,17	6,9,3	8,11	9	22	14,2,15,2	17	11	14,17	X	10,11	20,24
23	SNU-2699BL	13,16	29,30	11,12	9,10	15,17	7,9	8,9	12	18	13,2,14	17,18	8	13,15	X.Y	11,12	21
24	SNU-2706.1BL	12,16	31,2	10,11	11,12	15,17	7	8,11	11,13	19,24	13,2,16,2	14,17	10,11	14,19	X	11,13	18,22
25	SNU-2740BL	13	30,2,32	11,12	12	15,17	7,9	8,10	11,12	17,19	13,14	18	8,11	19,25	X.Y	11,14,15	17,23,2
26	SNU-3048BL	11,12	29,31	10,12	10,11	15,16	7,9	10,11	11,12	18,23	13,14	17	11	15	X	11,12	22,23

A single round of PCR amplified 15 short tandem repeat markers and an amelogenin gender-determining marker at loci containing highly polymorphic microsatellite marker. DNA profiling of each cell lines was identified by DNA characteristics of each cell lines. As a result, we confirmed that all twenty-six LCLs are different origin of cancer patients.

### Identification of lymphocytes stained by wright stain

Twenty-six LCLs were stained by wright stain solution and through staining, it showed that cytoplasm and nucleus separated. Lymphocytes were identified by their large nucleus.



**Figure 3. B-lymphoblastoid cell lines stained by wright stain.** (A) Whole blood, white arrow represents neutrophil, black arrow represents eosinophil and gray arrow represents lymphocyte. (B) Lymphoblastoid cell lines. The same cell lines but differently segmented lymphocyte nuclei were observed and sometimes monocytes with high cytoplasmic ratio were also observed. Scale bar is 10  $\mu$ m.

**Table 3. PCR and RT-PCR primer sequences**

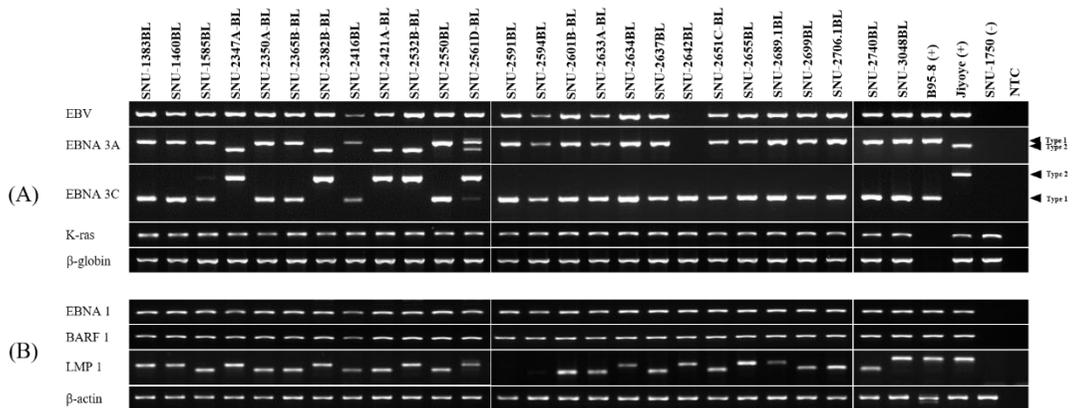
Type	Sequence	Annealing temperature & Cycle	Ref.
EBV	F 5'-CTT GGA GAC AGG CTT AAC CAG ACT CA-3'	60°C, 30	(8)
	R 5'-CCA TGG CTG CAC CGA TGA AAG TTA T-3'		
EBNA 3A	F 5'-GAA ACC AAG ACC AGA GGT CC-3'	60°C, 30	(10)
	R 5'-TCC CAG GGC CGG ACA ATA GG-3'		
EBNA 3C	F 5'-AGA AGG GGA GCG TGT GTT GT-3'	61°C, 30	(10)
	R 5'-GGC TCG TTT TTG ACG TCG GC-3'		
EBNA 1	F 5'-AGC AGG GGT GAT GAA AAC AG-3'	60°C, 40	(11)
	R 5'-CAG GCA GCA TCC CTG ATA TT-3'		
BARF 1	F 5'-GGC TGT CAC CGC TTT CTT GG-3'	65°C, 35	(12, 13)
	R 5'-AGG TGT TGG CAC TTC TGT GG-3'		
LMP 1	F 5'-CGG AAG AGG TGG AAA ACA AA-3'	55°C, 35	(14)
	R 5'-GTG GGG GTC GTC ATC ATC TC-3'		

**Detection of latent genes from EBV positive B-lymphoblastoid cell lines by PCR analysis**

twenty-six LCLs $\beta$  (Table 3)s were (Figure 4) The EBV primer shown that which cell lines were EBV positive cell lines or EBV negative cell lines and p (16). Twenty-five LCLs were confirmed that EBV except for SNU-2642BL. Also, was conducted by EBNA 3 (17) (18) I have confirmed that EBNA 3A and EBNA 3C showed different sizes depending on the type of EBV.

**Detection of latent genes from EBV positive B-lymphoblastoid cell lines by RT-PCR analysis** Analysis the Epstein-Barr virus mRNA expression level by RT-PCR. $\beta$  and positive control amplifications of DNAs from (Table 3)

primers for (Figure 4) EBNA 1 primers amplified a 167 bp fragment, BARF 1 primers amplified a 203 bp fragment, and LMP 1 primers amplified a 123 bp or 161 bp fragment (19-22). I confirmed the expression of each gene in all twenty-six LCLs. All twenty-six LCLs showed EBNA 1, BARF 1 and twenty-four LCLs showed LMP 1 except SNU-2591BL and SNU-2594BL.



**Figure 4. PCR analysis of the Epstein-Barr virus (EBV) gene from B-lymphoblastoid cell lines and analysis of the Epstein-Barr virus (EBV) mRNA expression level by RT-PCR.** Twenty-six LCLs were screened by PCR and RT-PCR analysis. (A) Internal PCR by K-ras and  $\beta$ -globin, EBV specific PCR by EBV, EBNA 3A and EBNA 3C. Lane: 1 and 26, amplification of DNAs from LCLs; 27 and 28, control amplifications of DNAs from type 1 B95-8 and type 2 Jiyoye EBV, respectively; 29, control amplification with no infected by EBV; 30, control amplification with primers and no added DNA. (B) Control PCR by  $\beta$ -actin, EBV mRNA expression level was analyzed by EBNA 1 (40 cycles), BARF 1 (35 cycles) and LMP 1 (35 cycles).

Also, because using 17 well gels, I could not load all the twenty-six LCLs at once with containing controls. Therefore, the cell lines were divided and loaded, and only the controls were marked at the end.

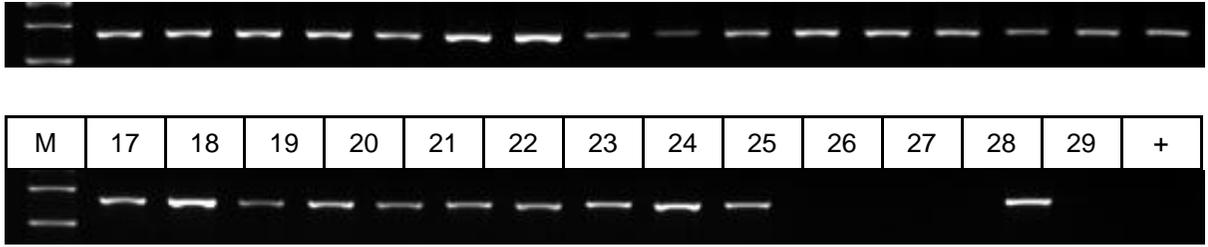
**Table 4. Type classification of EBV positive B-lymphoblastoid cell lines by PCR analysis**

NO	Cell line	EBV	EBNA 3A	EBNA 3C
1	SNU-1383BL	O	Type 1	Type 1
2	SNU-1460BL	O	Type 1	Type 1
3	SNU-1585BL	O	Type 1	Type 1+Type 2
4	SNU-2347A-BL	O	Type 2	Type 2
5	SNU-2350A-BL	O	Type 1	Type 1
6	SNU-2365B-BL	O	Type 1	Type 1
7	SNU-2382B-BL	O	Type 2	Type 2
8	SNU-2416BL	O	Type 1	Type 1
9	SNU-2421A-BL	O	Type 2	Type 2
10	SNU-2532B-BL	O	Type 2	Type 2
11	SNU-2550-BL	O	Type 1	Type 1
12	SNU-2561D-BL	O	Type 1+Type 2	Type 1+Type 2
13	SNU-2591BL	O	Type 1	Type 1
14	SNU-2594BL	O	Type 1	Type 1
15	SNU-2601B-BL	O	Type 1	Type 1
16	SNU-2633A-BL	O	Type 1	Type 1
17	SNU-2634BL	O	Type 1	Type 1
18	SNU-2637-BL	O	Type 1	Type 1
19	SNU-2642BL	△	Type 1	Type 1
20	SNU-2651C-BL	O	Type 1	Type 1
21	SNU-2655BL	O	Type 1	Type 1
22	SNU-2689.1BL	O	Type 1	Type 1
23	SNU-2699-BL	O	Type 1	Type 1
24	SNU-2706.1-BL	O	Type 1	Type 1
25	SNU-2740BL	O	Type 1	Type 1
26	SNU-3048BL	O	Type 1	Type 1

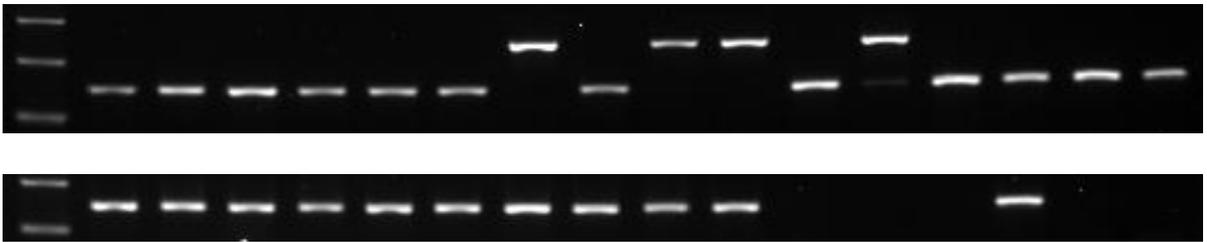
Twenty LCLs were type 1, and SNU-1585BL, SNU-2347A-BL, SNU-2382B-BL, SNU-2421A-BL, SNU-2532B-BL and SNU-2561D-BL were type 2. In case of SNU-1585BL and SNU-2561D-BL, both type 1 and type 2 were shown..

### 1) EBV F/R

M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
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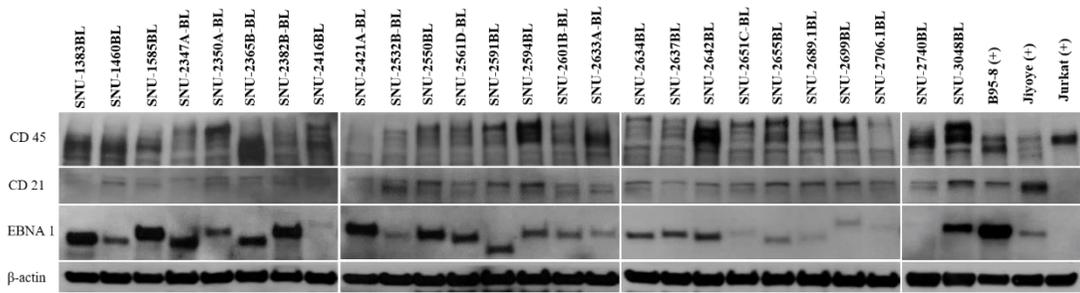
## 2) EBNA3C F/R



## EBV positive B-lymphoblastoid cell lines express the EBV latent protein EBNA

### 1, lymphocyte and B cell marker

EBNA 1 protein expression level was analyzed by Western blot analysis in twenty-six LCLs. Also, CD45 (leukocyte common antigen), CD21 (complement type 2, CR2) were identified by western blot. Each protein expression level was detected from the same membrane after being washed with 2.0% skim milk. As a result, the expression of CD45 (180-220 kDa) and CD21 (145 kDa) in all LCLs was confirmed and EBNA 1 had a different size band for each LCLs.



**Figure 5. Western blot analysis of lymphocyte, B cell marker and EBV latent protein EBNA 1.** Western blot analysis was performed to monitor the EBV-induced proteins, such as EBNA 1. CD45, a common leukocyte marker, and CD21, complement type 2, were used to confirm that LCLs were B cells. Positive controls for CD45 and CD21 were B95-8 (type 1 EBV), Jiyoye (type 2 EBV) and Jurkat cells (immortalized line of human T lymphocyte cell). Whereas, in the case of EBNA1, B95-8 and Jiyoye were positive controls, and in immortalized line of human T lymphocyte cells, Jurkat cells were negative controls. Also, because using 17 well gels, I could not load all the twenty-six LCLs at once with containing controls. Therefore, the cell lines were divided and loaded, and only the controls were marked at the end.



**Analysis of cell marker expression in Epstein-Barr virus (EBV) positive B-lymphoblastoid cell lines by Flow cytometry (FACS).**To identify the cell population, FACS analysis was conducted using pan leukocyte (CD45), T-cell subset (CD4 and CD8), B cell (CD19 and CD20), NK cell (CD56) and monocyte/macrophage (CD68), Pan-cytokeratin (epithelial cell), PD-L1 (programmed death-ligand 1) and PD-1 (programmed death 1) markers in Table 4 (A). Also in Table 4 (B) was subdivided into B cell by CD27 (memory B cell) and IgD (naïve B cell). Twenty-two LCLs showed B cell population, SNU-1585BL, SNU-2601B-BL and SNU-3048BL showed T cell and SNU-2651C-BL showed NK cell population. SNU-1585BL and SNU-3048BL were confirmed that not only the B cell

population but also the T cell population. However, as passage increased, the population of T cells decreased in most LCLs, while the population of B cells tended to increase (Table 5 (A)). In the case of SNU-2651C-BL, the population of NK cells was increased as passage increases.

In all twenty-six LCLs, PD-L1 and pan-cytokeratin showed a population of less than 10%, whereas SNU-2347A-BL, SNU-2421A-BL, SNU-2532B-BL, SNU-2651C-BL, SNU-2706.1-BL and SNU-2740BL showed more than 60% of PD-1 population.

In Table 5 (B), B cell was further subdivided based on the predominance of B cell population in Table 5 (A). The results of CD27, IgD in the positive region of CD19 and CD20 were observed. CD19 was expressed from pre-B cells stage to plasmablast and early plasma cells, and CD20 was expressed only in mature B cell. Therefore, to analyze B cell differentiation, CD19 and CD20 were stained for each LCLs.

When the B cell was subdivided, the percentage of CD27, a memory B cell marker, was higher than that of IgD in some LCLs. Twenty-four LCLs were differentiated from naïve B cells to memory B cells but SNU-2642BL and SNU-2706.1-BL were in the naïve B cell stage and SNU-1460BL and SNU-2532B-BL for non-switched memory cells and SNU-2365B-BL, SNU-2382B-BL, SNU-2594BL, SNU-2601B-BL, SNU-2633A-BL, SNU-2634BL and SNU-3048BL for class-switched memory cells.

In addition, in case of anti-CD68, there was no significant difference in

permeabilization and non-permeabilization results in LCLs therefore only the results without permeabilization were showed.

**Table 5 (A). Result of Flow cytometry stained by B cell, T cell, NK cell, monocyte/macrophage, epithelial, PD-L1 and PD-1 markers**

No.	Cell line	CD45	CD4	CD8	CD19	CD20	CD56	CD68	Pan-cytokeratin	PD-L1	PD-1
1	SNU-1383BL	95.2	2.8	0.3	96.1	94.6	0.0	0.0	0.4	2.1	2.7, 43.2
2	SNU-1460BL	99.3, 63.1	5.8	0.7	99.0	98.3, 73.1	0.0	0.0	0.2	1.2	1.7, 58.1
3	SNU-1585BL	96.5	38.8, 0.5	1.1	60.6, 20.2	91.6, 50.8	0.0	0.0	0.5	4.4	3.5
4	SNU-2347A-BL	57.0, 93.9	3	1.1	75.0, 57.3	91.5	0.0	0.0	1.6	0.3	40.7, 71.6
5	SNU-2350A-BL	98.6	5.2	0.9	73.4	99.8	0.0	0.0	7.0	0.5	31.3, 55.8
6	SNU-2365B-BL	98.5	3.3	0.4	96.4, 68.2	90.9, 62.2	0.0	0.0	0.0	1.2	1.6, 15.5
7	SNU-2382B-BL	97.4, 58.6	2.5	1.2	72.3, 54.8	91.1, 65.0	0.0	0.0	0.3	3.8	3.7, 23.6
8	SNU-2416BL	96.0	0.0	0.8	66.5	88.7, 65.4	0.3	0.0	0.3	2.8	3.1, 39.3
9	SNU-2421A-BL	83.9	0.0	0.4	73.7, 36.2	86.1, 38.4	0.0	0.0	0.2	3.1	4.2, 75.4
10	SNU-2532B-BL	80.9	0.6	0.5	66.3, 93.4	92.5	0.0	0.0	0.7	1.4	4.1, 68.1
11	SNU-2550-BL	99.7, 64.9	8.1, 0.6	3.5, 0	95.8, 77.3	97.6, 50.2	0.1	0.0	0.1	0.5	0.3, 24.0
12	SNU-2561D-BL	85.3	2.5	1.2	82.4	86.5	0.1	0.0	6.6	0.5	55.3
13	SNU-2591BL	94.9	8.2	1.1	6.9, 28.5	78.1	0.0	0.0	0.5	0.1	7.1, 29.6
14	SNU-2594BL	83.9	0.2	2.0	80.5, 40.9	75.5	0.0	0.0	1.2	0.4	23.3, 48.6
15	SNU-2601B-BL	99.2, 56.3	10.1, 3.9	0.7	95.4	99.5	0.2	0.0	4.6	1.2	27.2
16	SNU-2633A-BL	71.5, 99.8	0.4, 2.1	0.6	41.0	40.5, 73.6	0.0	0.0	4.4	1.2	42.0, 15.6
17	SNU-2634BL	67.8, 99.4	0.8	0.9	83.5	99.4	0.0	0.1	0.8	0.3	16.4, 55.6
18	SNU-2637-BL	96.2	0.8	0.0	25.6, 72.6	85.7	0.0	0.0	4.2	0.2	4.0, 28.6
19	SNU-2642BL	99.3	3, 6.6	0.3	35.9, 58.4	68.8, 98.0	0.0	0.0	0.7	0.5	14.2
20	SNU-2651C-BL	93.6	6.5	0.2	80.8	99.3	19.2	0.0	0.4	0.3	26.2, 75.5
21	SNU-2655BL	89.6	0.4	0.5	74.0	91.8	0.0	0.0	0.2	0.1	4.4, 64.9
22	SNU-2689.1BL	95.8, 50.0	2.4	1.2	81.3, 58.9	77.2	0.0	0.0	0.4	0.1	5.5, 52.6
23	SNU-2699-BL	98.9, 55.9	4.5	0.3	48.4	86.6	0.0	0.0	1.2	0.5	7.4, 51.6
24	SNU-2706.1-BL	60.6, 31.5	1.3	0.4	21.1, 77.3	78.0, 90.1	0.0	0.0	1.9	0.6	34.2, 74.9
25	SNU-2740BL	99.2, 38.8	1.7	0.3	35.0, 61.7	31.0	0.0	0.0	0.5	0.3	80.7, 42.7
26	SNU-3048BL	94.1, 52.1	10.1, 0.4	0.4	50.0, 83.1	95.2	0.0	0.0	1.6	0.1	21.6, 9.7

**Table 5 (A). Result of Flow cytometry stained by B cell, T cell, NK cell, monocyte, epithelial, PD-L1 and PD-1 markers**

The data were processed in three repeats, only the percent difference according to cell passage was recorded separately.

**Table 5 (B). Result of Flow cytometry stained by B cell markers**

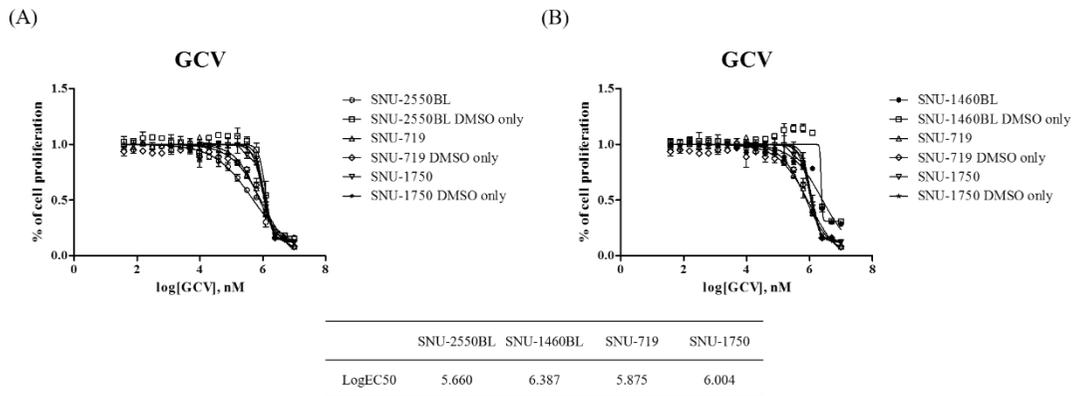
No.	Cell line	CD45	CD19		CD20	
			CD27	IgD	CD27	IgD
1	SNU-1383BL	95.0	0.8	0.0	0.8	0.1
2	SNU-1460BL	68.0, 99.5	2.5, 28.7	3.6, 9.0	2.6, 24.2	3.6, 7.4
3	SNU-1585BL	95.3, 32.9	0.3	0.1	0.2	0.1
4	SNU-2347A-BL	64.9, 42.9	0.8	0.0	5.4	0.0
5	SNU-2350A-BL	68.9, 92.7	0.4, 22.1	0.1	0.5, 24.3	0.1
6	SNU-2365B-BL	95.2, 55.4	10.8, 0.4	0.0	15.7, 1.8	0.0
7	SNU-2382B-BL	90.7, 30.6	32.7, 9.2	0.1	39.5, 18.7	0.1
8	SNU-2416BL	91.0	8.5, 3.7	0.1	8.5, 4.2	0.0
9	SNU-2421A-BL	71.3, 12.9	0.1	0.1	0.0	0.0
10	SNU-2532B-BL	86.8, 68.9	11.9, 1.9	33.2, 6.8	12.6, 3.8	32.3, 5.2
11	SNU-2550-BL	40.6, 78.7	0.1	0.1	0.0	0.1
12	SNU-2561D-BL	59.0, 79.0	0.6	0.2	2.6	0.2
13	SNU-2591BL	97.3, 68.8	0.0	0.0	0.0	0.0
14	SNU-2594BL	62.8, 88.7	10.5, 4.5	0.1	14.7, 3.6	0.1
15	SNU-2601B-BL	99.8, 28.0	53.2, 4.1	0.1	51.4, 8.5	0.1
16	SNU-2633A-BL	52.1, 98.1	0.0, 12.7	0.0	0.1, 17.0	0.0
17	SNU-2634BL	69.6, 97.7	0.2, 21.9	0.0	0.0, 12.8	0.0
18	SNU-2637-BL	82.5, 61.7	3.1, 0.8	0.0	13.4, 2.7	0.0
19	SNU-2642BL	95.8	0.0	0.0, 12.5	0.0	0.0, 15.5
20	SNU-2651C-BL	91.7	6.3	0.1	3.0	0.1
21	SNU-2655BL	93.1	0.1	0.1	0.0	0.0
22	SNU-2689.1BL	86.7, 34.7	0.0	0.0	0.1	0.0
23	SNU-2699-BL	96.2, 49.2	0.4	0.0	0.3	0.0
24	SNU-2706.1-BL	46.2, 39.2	0.0, 4.7	2.8, 62.8	0.1, 8.6	0.1, 51.1
25	SNU-2740BL	97.6, 49.8	0.2	0.0	0.1	0.0
26	SNU-3048BL	79.4, 40.2	8.4, 15.4	0.0	4.6, 18.6	0.0

**Table 5 (B). Result of Flow cytometry stained by B cell markers**

The data were processed in three repeats, only the percent difference according to cell passage was recorded separately.

### Drug sensitivity analysis

Drug sensitivity of LCLs were measured by monoclonal antibody-based drug such as Ganciclovir (GCV). LogEC50 of SNU-2550BL was 5.660 and LogEC50 of SNU-1460BL was 6.387. SNU-2550BL and SNU-1460BL represented as sensitivity to GCV.

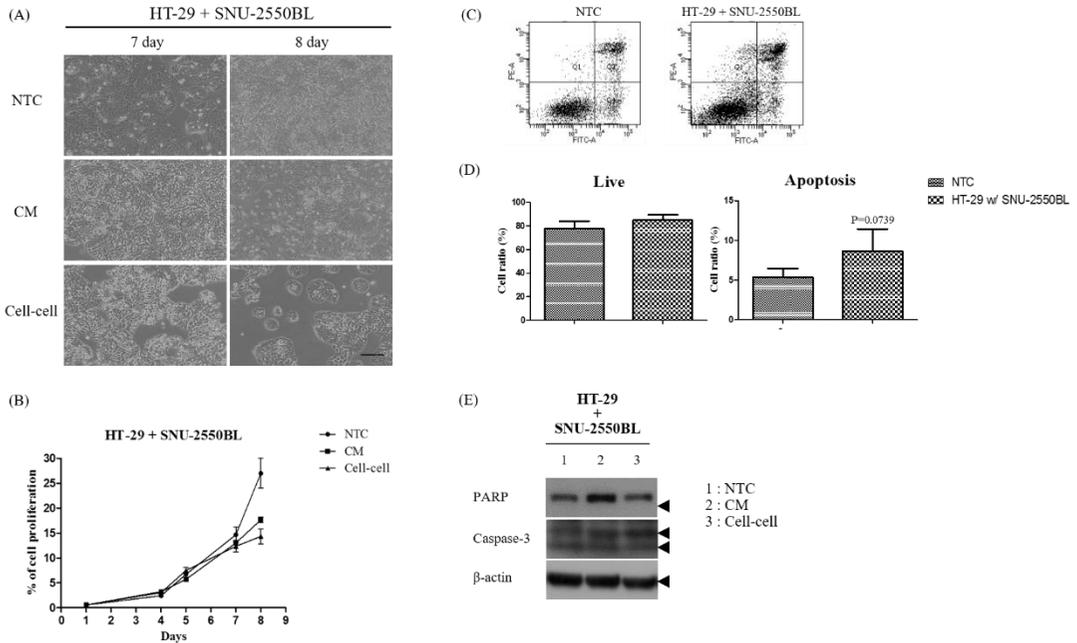


**Figure 6. Drug sensitivity analysis.** Ganciclovir (GCV) was treated to LCL at the highest concentration of  $1 \times 10^4$  nM. SNU-719 is a gastric cancer cell line which is a positive control for EBV and SNU-1750 is a gastric cancer cell line which is a negative control for EBV. (A) The sensitivity to GCV of SNU-2550BL, SNU-2550BL DMSO only represented negative control (Dimethyl sulfoxide). (B) The sensitivity to GCV of SNU-1460BL, SNU-1460BL DMSO only represented negative control (Dimethyl sulfoxide). Both the result of SNU-2550BL and SNU-1460BL were sensitivity to GCV.

**Co-culture analysis of Epstein-Barr virus (EBV) positive B-lymphoblastoid cell**

### **line with cancer cell line induces apoptosis in cancer cell**

To confirm whether twenty-six LCLs induce apoptosis of cancer cell, I co-cultured LCLs with cancer cell line. The experiment was divided into three groups, co-culture of SNU-2550 cancer cell and SNU-2550BL which is paired with cancer cell line showed that tremendous anticancer effect of LCL. In order to confirm the anticancer effect of LCL itself, I co-cultured with HT-29, another cancer cell and I have also identified that another LCL, SNU-1460BL, has an anticancer effect. In order to show more reliable results, counting assay was performed. As a result, co-culture results of SNU-2550 cancer cell line and paired LCL, SNU-2550BL showed a clearer cancer cell death than other sets. Based on these results, I conducted an apoptosis assay using Annexin V to determine whether LCL actually induces apoptosis in cancer cells. After co-culture, apoptosis assay was performed after 5 hr. As a result, apoptosis tended to increase with co-culture of SNU-2550BL and SNU-1460BL compared to NTC (SNU-2550 and HT-29 cancer cell only). Also, I confirmed protein expression level with poly (ADP-ribose) polymerase (PARP) and caspase-3, a DNA damage signal antibody, to determine if they caused damage to the DNA and resulted in apoptosis. In the case of caspase-3, there was no difference in expression between the co-culture sample and the non-co-culture sample. However, in the case of PARP, the expression of cleaved PARP was found to be different between the co-culture sample and the non-co-cultured sample (SNU-2550 co-cultured with SNU-2550BL).



**Figure 7. Co-culture analysis of Epstein-Barr virus (EBV) positive B-lymphocyte cell line with cancer cell line.**

Twenty-six LCLs were co-cultured with cancer cell line. Negative control (NTC) as SNU-2550 cancer cell or HT-29 cancer cell only, Conditioned media (CM) as LCLs were co-cultured with cancer cell line using filter dish and Cell-cell interaction (Cell-cell) as same as CM not using filter dish but dish. CM signified conditioned media of LCLs and Cell-cell intended to interaction between LCLs and cancer cell line. Scale bar is 100  $\mu$ m. (A) Changes in morphology and cell confluency over time. (B) Counting result of time-dependent process. (C) Apoptosis assay with Annexin V antibody conjugated with Alexa 488. Q1 means death (unrelated apoptosis), Q2 upper means death, down means late apoptosis, Q3 means live cell and Q4 means early apoptosis (23). (D) The percentage of live cells (left panel), and the percent of both

early and late apoptosis, dead cells (right panel). (E) Expression of cleaved PARP and caspase-3. Results represent means  $\pm$  standard deviation. The asterisks indicate statistical significance.  $P < 0.05$ , and  $P < 0.01$ .

## Discussion

In this study, EBV positive B-lymphoblastoid cell lines naturally derived from cancer patients. It was focused on analyzing the characteristics of LCLs and ultimately treating EBV-related malignancies using these cell lines.

When EBV infects host DNA, it goes into pre-latency state, then becomes latency state, and proceeds to lytic cycle to produce viral particles. Lymphoblastoid cell lines correspond to latency type-3, where latent proteins of LCLs were expressed. Epstein-Barr nuclear antigen 1 (EBNA 1) was expressed in all EBV-related malignancies and it plays critical roles in the maintenance, replication and transcription of the EBV genome (24). BamH1-A Reading Frame-1 (BARF 1) and Latent Membrane Protein-1 (LMP 1) also known as oncogenes. The BARF 1 protein can complex *in vitro* with colony-stimulating factor 1, resulting in the inhibition of macrophage-activation, and can also inhibit the secretion of IFN- $\alpha$  in EBV-infected B cells. BARF 1 was therefore involved not only in oncogenic development, but also in immunomodulation (25). LMP 1 was essential for B cell immortalization and functional homologue of tumor necrosis factor and mediates signaling through the nuclear factor- $\kappa$ B pathway, mimicking CD40 receptor signaling. In conclusion, EBNA 1 and LMP 1 latent proteins related to EBV oncogene were expressed in LCLs.

Also, the expression of the LCLs was confirmed by western blot with CD45

(leukocyte common antigen), CD21 (complement type2, CR2) and EBNA 1 (Figure 5). As a result, the expression of CD45 and CD21 in all LCLs was confirmed and EBNA 1 had a different size band for each LCLs. EBNA 1 acts through sequence-specific binding to the plasmid origin of viral replication (oriP) within the viral episome. The oriP has four EBNA 1 binding sites where replication was initiated as well as a 20-site repeat segment which also enhances the presence of the protein. So the differences in the apparent molecular weight of EBNA 1 detected in these LCLs was likely attributable to variations in the internal repeat region 3 within the EBNA 1 coding region (26). B cell have CD21 (complement receptor 2, CR2) on their surfaces, allowing the complement system to play a role in B-cell activation and maturation. Also, CD21 has been shown to interact with CD19 and plays an important role in enhancing mature B cell responses to foreign antigens (27). Therefore, my data was confirmed that CD21, which plays an important role in B cell activation and maturation, was expressed in all LCLs (Figure 5).

Human B cells begin to differentiate from bone marrow, progenitor B cell (pro-B cell) is differentiated into precursor B cell (pre-B cell) and immature B cell, followed by mature naïve B cell, memory B cell, and plasma cell or plasmablast. Typically, mature naïve B cell is identified by IgD expression, while memory B cell is identified by expression of CD27. When CD27 and IgD are simultaneously expressed, they signify non-switched memory B cell, if CD27 is expressed but IgD is not expressed, it means class-switched memory B cell (28, 29). My data demonstrated that all

twenty-six LCLs corresponded to B lymphocytes. Analysis of B cell development stage revealed that most of LCLs were memory B cells. In case of CD27-IgD-, were considered to be pro-B cell (early, late) or pre-B cell (large, small) or immature pre-B cell stage.

Tumors are characterized by uncontrolled cell growth and are known to express higher levels of thymidine kinase (TK) activity than normal tissue. TK is activated in the G1/S phase of the cell cycle, and its activity has been shown to correlate with the proliferative activity of tumor cells. Therefore, if the antiviral drug ganciclovir (GCV) targets tumor TK, it can inhibit tumor growth (30). Previous studies have shown that certain viruses induce the production and activity of cellular TK. I treated GCV to LCLs because I do not know whether TK is expressed in LCLs. As a result, it was found that LCLs were sensitive to GCV (Figure 6). If when EBV thymidine kinase acts actively in LCLs or insertion of TK gene in LCLs through cloning, it will be demonstrated that GCV target EBV (31). However, conventional therapies do not selectively target infected cells, and side effects are common. In addition, no currently licensed antiviral drugs are able to eliminate cells in which the virus is latent. Therefore, targeting LCLs with EBV virus in latent state, I think it would be benefit for screening of therapy (32-36).

The co-culture of cancer cells with LCLs showed a tendency to induce apoptosis in cancer cells, since viral and immune factors secreted by LCLs are not yet known, further studies are needed to identified what mechanisms of LCLs inhibit cancer cell

growth. Also, comparing the degree of apoptosis according to the reaction time, the result of apoptosis will be more accurate and by confirming the expression of Lactate dehydrogenase (LDH), a marker of cell damage, it can be proved that LCLs induce apoptosis in cancer cell.

Apoptosis is a process of programmed cell death that occurs in multicellular organisms. It was lead to characteristic cell change (morphology) and death. This change involved blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation as well as cleavage of poly (ADP-ribose) polymerase (PARP) (37-39). In addition, caspase-3, one of the last activated caspases, also appeared and sequential activation of caspase played a central role in the execution-phase of cell apoptosis. There are at least two molecular pathways leading to apoptosis (the death receptor pathway and mitochondrial pathway). However, even without caspase activation, caspase-independent cell death is still induced by stimuli such as cell shrinkage, cytoplasmic condensation, and loss of mitochondrial membrane integrity and exposure of phosphatidylserine (40). Thus, in my co-culture experiments, there was no difference in the expression of active caspase-3 between co-cultured and non-co-cultured. In this case, caspase-independent apoptosis is thought to cause cancer cell death.

In conclusion, I analyzed the type and characterization of twenty-six LCLs and confirmed the anticancer effect of this LCLs. Furthermore, since EBV, which have 90% of the world's total population and is in a latent state in asymptomatic, is highly

likely to cause cancer, further studies are needed to treat EBV-related cancers in the future.

## References

### 국문 초록

Herpesvirus과에 속하고 인간 herpesvirus 4 (HHV-4)라고도 불리는 Epstein-Barr virus (EBV)는 인간에서 가장 흔한 virus중에 하나이다. 이러한 virus는 세계 전 인구의 90%가 가지고 있으며, 대부분이 그 증상을 나타내진 않는다. 보통 스트레스를 받았거나 아프고, 면역시스템이 고장 났을 때 우세하게 나타나며, 이는 전염성 단핵증의 원인으로 잘 알려져 있고, 특정 암 (버킷 임파종, 호지킨 림프종 등) 과도 연관이 있다. 이러한 EBV 감염은 특정 자가면역질환과도 매우 연관이 높으며, 1년동안에 20만명의 암환자들이 EBV에 의해 발생된다는 것을 알 수 있다. 하지만 EBV 관련 암들을 치료하는 최적의 방법들에 대한 연구는 진행되고 있지 않아서 앞으로 많은 연구가 필요할 것으로 생각된다. 본 연구에서는 종양세포주 수립을 위한 세포 배양 중에, 조직 속에 포함된 EBV에 감염되었던 림프구가 체외에서 증식하여 수립된 EBV 양성 B 림프구 세포주들의 특성을 분석하기 위해서, 라이트 염색, 중합효소연쇄반응, 웨스턴블롯, 유동 세포 분석법 및 약제내성실험을 진행하였다. 그리고 EBV 양성 B 림프구 세포주를 이용하여서 다양한 EBV 관련 암들을 치료하기 위한 목적으로 암세포와의 공동배양을 실시하였다.

그 결과, EBV 양성 B 림프구 세포주임을 증명하였고, 대부분이 B 림프구 세포주였음을 확인하였다. 또한, virus를 표적으로 하여 EBV 관련 암들을 치료하는 Ganciclovir (GCV)를 EBV 양성 B 림프구 세포주에 처리함으로써 민감성 있는 결과를 도출해내었고, 화학약물의 독성으로 인한 부작용을 나타내는 항암제보다는 virus 자체를 표적으로 하는 GCV를 이용해서 다양한 EBV관련 악성종양을 집중적으로 치료하는데 효과가 있을 것이라고 생각된다. 그리고 EBV 양성 B림프구 세포주에서 분비되는 물질에 의해 암세포에 세포사멸을 유도하고 그로 인해 암세포의 성장을 저해한다는 것을 증명하기 위해 EBV 양성 B 림프구 세포주와 암세포와의 공동배양을 실시하였다. 그 결과, 같은 한 환자에서 유래된 EBV 양성 B 림프구 세포주와 암세포와의 공동배양이 다른 환자에서 유래된 EBV 양성 B 림프구 세포주와 암세포와의 공동배양을 한 것보다 확실하게 세포사멸을 유도한다는 결과를 도출해내었다. 하지만 어떠한 기작으로써 암세포에 세포사멸을

유도하는지는 더 많은 연구가 필요할 것으로 생각된다.

결론적으로, 본 연구에서는 암환자로부터 자연스럽게 수립이 된 EBV 양성 B 림프구 세포주들의 특성분석을 통해 증명해내었고, 공동 배양을 통해 EBV 양성 B 림프구 세포주들이 암세포에 세포사멸을 유도하고 그로 인해 암세포의 성장 저해를 초래한다는 것을 확인하였다. 하지만 세계 전 인구의 90%가 잠복상태로 가지고 있는 EBV는 암을 발생시킬 수 있는 가능성이 높은 존재이기 때문에 앞으로 EBV와 관련된 암을 치료하는데 더 많은 연구가 필요할 것으로 생각된다.

주요어 : Epstein-Barr Virus (EBV), B 림프구, 잠복유전자, 공동배양, 세포사멸

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