APOPTOSIS-RESISTANT DIFFERENCE BETWEEN EBV-BHRF1 GENE POSITIVE AND NEGATIVE CELL LINES AGAINST 3 KINDS OF APOPTOSIS INDUCING FACTORS

ZHU Zhen-yu 朱振宇, DENG Yi-qun 邓诣群, HUNANG Bi-jun 黄必军
ZHANG Qing-xiu 张清秀, DAI Ke-sheng 戴克胜, MA Jian-quan 马涧泉

Department of Biochemistry, Sun Yat-sen University of Medical Sciences, Guangzhou 510089

ABSTACT

Objective: To study the apoptosis-resistant difference between EBV-BHRF1 gene positive and negative cell lines against 3 kinds of apoptosis inducing factors. Methods: These cell lines were cultured in (1) the absence of fetal calf serum (FCS) for 48 h, (2) 43°C for 10 min, and (3) 2×10^{-6} mol/L dexamethasone for 24 h. Polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), and apoptosis inducing and detecting methods were used. Results: EBV-BHRF1 gene fragment was amplified from $B_{95\text{-}8}$, Raji and SUNE-1 cell lines with PCR technique, while K_{562} and YAC cell lines were negative. The results against 3 kinds of apoptosis inducing factors from electron microscopy and agarose gel electrophoresis indicated that apoptosis appeared in BHRF1 gene-negative cell lines while apoptosis were normal in BHRF1 gene-positive cell lines. Conclusion: EBV-BHRF1 gene is one of the important factors in suppressing its host cell's apoptosis.

Key words: Apoptosis, Dexamethasone, EBV-BHRF1 gene, Polymerase chain reaction, RT-PCR.

Apoptosis, also known as programmed cell death (PCD), is a kind of particular cell death.[1] Negative regulator of apoptosis may play an important role in the etiology of a variety of tumors. Inducting tumor cell apoptosis is one of the major targets for various treatments.[2] Bcl-2 protein could inhibit cell apoptosis induced by lots of factors. It is very important in gene regulating process of apoptosis.[3] Epstein-Barr virus (EBV) has been linked to the etiology, development and metastasis of nasopharyngal carcinoma (NPC). EBV-coded BHRE1 (EBV-BHRF1) protein which is 38% homologous of bcl-2, is also located in similar positions as bcl-2 does in cells.[4] There is no report shows tumor cells containing BHRF1 gene have stronger apoptosis-resistant against apoptosis inducing factors. In this study, we investigated the apoptosis-resistant difference between EBV-BHRF1 gene positive (3 cell lines) and negative (2 cell lines) cell lines against 3 kinds of apoptosis inducing factors, to determine whether this gene product has the anti-apoptotic ability.

Accepted for Publication May 28, 1999
This work was supported by the Youth Science Research Foundation of Guangdong Province Science and Technology Commission, China (No. 950355).
Correspondence to: ZHU Zhen-yu; Department of Biochemistry, Sun Yat-sen University of Medical Sciences, 74 zhongshan Road II, Guangzhou 510089, China; Fax: (0086-20-87331679 (China), 412-624-7736 (USA); E-mail: zhuzy@pitt.edu/zhenyuzhu@hot mail.com

MATERIALS AND METHODS

Cell Lines

$B_{95\text{-}8}$, Raji and low differential NPC cell line (SUNE-1) were provided by Cancer Institute of Sun Yat-sen University of Medical Sciences (SUMS); K_{562}, YAC cell lines were provided by Department of Pathophysiology of SUMS.

EBV-BHRF1 Specific Primers

Designed according to the EBV whole sequence (172, 282 bp, Gene Bank).[5] Primer I:5'-AGCAAGA-TGGCCTATTCAAC-3; Primer II:5'-AATGTCGACAT-AAGTGTGTTCCT-3'.

RPMI-1640 Medium, PCR, RT-PCR Kit (Superscrip ™, One-step ™, RT-PCR System) and 1 Kb DNA Ladder

They were all GIBCOBRL products.

Template DNAs Isolation and PCR Amplification

All cell lines were maintained in RPMI-1640 complete media, cultured and harvested as we described before.[6] Lysis each cell line to prepare template DNA.

Amplified the EBV-BHRF1 this fragment was with regular PCR.

RNA Preparation

Total cellular RNA of each cell line was isolated from 1×10^6 cells. Put 1 ml ultraspec ™ RNA kit (Biotecx Labotatories Inc. Houston, Texas, USA) to the cells, add 0.2 ml of chloroform, shake vigorously for 15 seconds, centrifuge at 12,000 g for 15' at 4°C. Carefully transfer 80% of the aqueous phase to a fresh tube while taking care not to disturb the interphase. Add equal volume of isopropanol, centrifuge samples at 12,000 g for 10' at 4°C. Remove the supernant and wash RNA pellet twice with 1 ml 75% ethanol by vortexing and subsequent centrifugation for 5' at 7,500 g at 4°C. Dissolve the RNA pellet in 30 μl of 0.1% DEPC treated water. The final preparation of undegraded RNA is free of DNA and protein and has A_{260}/A_{280} ratio between 1.8–2.0.

RT-PCR (one-step)

Using β-actin specific primers as internal control. Add the following components to 0.2 ml microcentrifuge tubes placed on ice (Table 1).

Gently mix and make sure that all the components are at the bottom of the amplification tube using following programes to finish RT-PCR:

(1) cDNA synthesis and pre-denaturation (perform 1 cycle): 50°C for 30' → 94°C for 2'; (2) PCR amplification (94°C, 15'' → 60°C, 30'' → 72°C, 60''; 25 cycles) →72°C for 10'.

Inducing of Apoptosis[7]

Each cell line was treated with the following condition, respectively: with the absence of fetal calf serum (FCS) for 48 h; 43°C for 10 min; and 2×10^{-6} mol/L dexamethasone (DEX) for 24 h.

Table 1. Added components for RT-PCR

Components	Volume	Final concentration
2 × reaction mix	25 μl	1 ×
Template RNA	x μl	100 ng
BHRF1 primer I (10 μM)	1 μl	0.2 μM
BHRF1 Primer II (10 μM)	1 μl	0.2 μM
β-actin primer I (2 μM)	1 μl	40 nM
β-actin primer II (2 μM)	1 μl	40 nM
RT-Taq mix	1 μl	
Autoclaved distilled water to 50 μl		

Detecting of Apoptosis

Treated cells were detected with electron microscopy; DNA fragment isolation and agarose gel electrophoresis were performed as described previously.[7,8]

RESULTS

The Results of EBV-BHRF1 PCR

Using template DNAs isolated from 5 kinds of cells lines, EBV-BHRF1 specific 592 bp fragment was amplificated from B_{95_8}, Raji and SUNE-1 cell lines, while K_{562} and YAC cell lines were negative (Figure 1).

EBV-BHRF1 RT-PCR Results

Using template RNAs isolated from untreated five cell lines, RT-PCR results showed that no EBV-BHRF1 specific band but just β -actin band (Figure 1 did not show). In cell lines treated with one of the three kinds of apoptosis inducing factors, RT-PCR results showed that B_{95_8}, Raji and SUNE1 cell lines had EBV-BHRF1 specific band (592 bp), while YAC and K_{562} were still negative. All cell lines contained β-actin specific band (346 bp) (Figure 2).

The Results of DNA Fragment Assay

After treatment with one of 3 kinds of apoptosis inducing factors, DNAs isolated from cells containing EBV-BHRF1 gene (B_{95_8}, Raji and SUNE-1) were entirely as normal, while those isolated from EBV-BHRE1 negative cells (K_{562} and YAC) showed typical oligonucleosomal ladder pattern of apoptosis DNA fragments which were 185 bp or its multiples (Figure 3).

The Results of Electron Microscopy

After treatment with one of 3 apoptosis inducing factors, electron microscopy showed that K_{562} and YAC cells chromatin is condensed, but their mitochondrial and cell membrane were preserved entirely (Figure 4), while B_{95_8}, Raji and SUNE-1 cell structures were nearly normal.

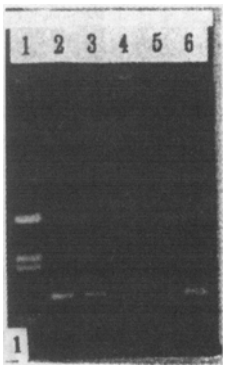

Fig. 1. 1.2% agarose gel electrophoresis of EBV-BHRF1 PCR products using template DNAs isolated from 5 cell lines.

Lane 1: DNA Marker: PBR322/Bst NI (1857, 1060, 929, 383, and 121 bp bands)

Lane 2–6: template DNAs isolated from B_{95-8}, Raji, K_{562}, YAC and SUNE-1 cell line respectively.

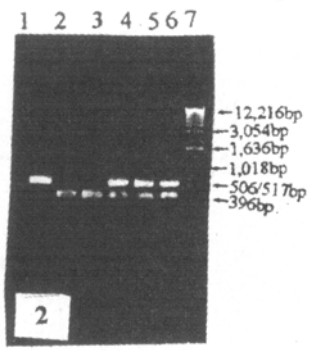

Fig. 2. 1.2% agarose gel electophoresis of EBV-BHRF1 PCR products using template RNAs isolated from 5 cell lines treated with 2×10^{-6} mol/L DEX for 24 h.

Lane 1: positive control showed 592 bp band. EBV-BHRF1 PCR product using untreated B_{95-8} cell DNA as template.

Lane 2–6: RT-PCR products using RANs from K_{562}, YAC, B_{95-8}, Raji and SUNE-1 cells respectively.

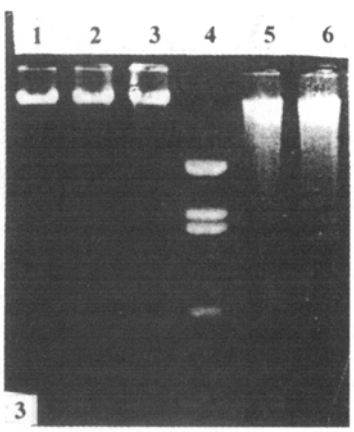

Fig. 3. 1.2% agarose gel electrophoresis of DNA extracted from 5 cell lines treated with 2×10^{-6} mol/L DEX for 24 h.

Lane 1–3: B_{95-8}, Raji and SUNE-1 cells line respectively;

Lane 4: DNA Marker, PBR322/Bst NI (1857, 1060, 929, 383, and 121 bp)

Lane 5, 6: K_{562} and YAC cell line respectively.

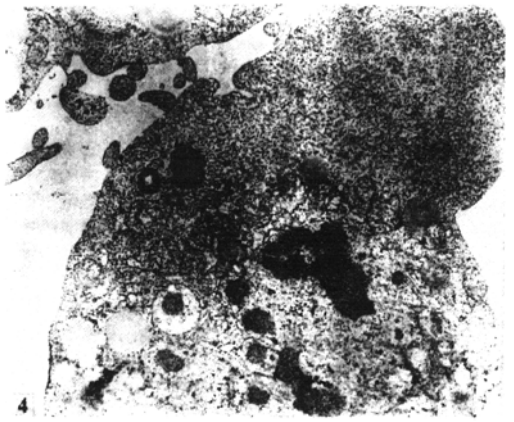

Fig. 4. Treated with 2×10^{-6} mol/L DEX for 24 h. Chromatin of the YAC cell is condensed, but its mitrochondrial and cell membrane were preserved entirely under electron microscopy.

All those results indicated that EBV-BHRF1 positive cell lines had stronger apoptosis-resistant ability than negative cell lines against 3 kinds of apoptosis inducing factors.

DISCUSSION

Apoptosis is a particular cell death. It plays important roles during cell growth differentiation and transformation. One of the most important reasons that most human tumors could occur, develop and metastasis is that tumor cells diminish or lose their response sensitivity to apoptotic inducing factors. EBV-BHRF1 gene has 38% homologue of bcl-2, its product also locates in a similar position as bcl-2 does in cells. There are reports which indicate that BHRF1 protein could also protect cells from PCD induced by a lot of factors.[4] Our previous report [9] showed that the recombinant EBV-BHRF1 product had the ability to protect nude mice spleen cells from DEX induced apoptosis. The results of this study indicates that expression of EBV-BHRF1 gene (mRNA) could protect its host cells from apoptosis induced by 3 kinds of factors. So EBV-BHRF1 is an important factor in suppressing its host cell's apoptosis.

REFERERCE

[1]. Hickman JA, Potten CS, Merritt A, et al. Apoptosis and cancer chemotherapy. Phil Trans R Soc Lond B 1994; 345:319.

[2]. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995; 267:1456.

[3]. Miyashita T, Reed JC. Bcl-2 oncoprotein blocks chemotherapy induced apoptosis in a human leukemia cell line. Blood 1993; 81:151.

[4]. Henderson S, Huen D, Rowe M, et al. Epstein–Barr virus-code BHRF1 protein, viral homologue of bcl-2, protects human B cells from programmed cell death. Proc Natl Acad Sci USA 1993; 90:8479.

[5]. Bcar R, Bankier AT, Biggin M, et al. DNA sequence and expression of the B_{95-8} Epstein-Barr virus genome. Nature 1984; 310:207.

[6]. Zhu Zhenyu, Yan Shirong, Huang Di, et al. Amplification, cloning, and identification of Epstein-Barr Virus-Dnase gene fragment. Acad J Sun Yat-sen Univ Med Sci 1995; 16:5.

[7]. Zhu Zhenyu, Pan Jingxuan, Zhao Qun, et al. Dexamethasone induces apotosis in nude mice spleen cells and affects natural killer cells' activity. Chin J Pathophysiol 1996; 12:113.

[8]. Pan Jingxuan, Zhu Zhenyu, Li Huiling, et al. The study of glucocorticoid-induced rat thymocyte programmed cell death. Chin J Pathophysiol 1996; 12:228.

[9]. Zhu Zhenyu, Xie Qiang, Pan Jingxuan, et al. Apotosis in nude mice spleen cells was inhibited by recombinant EBV-BHRF1 protien. Chin J Pathophysiol 1999; 5:122.