

시스플라틴 유도 세포사멸에서 Caspase 및 Bcl-2 Family 단백질에 의한 끝분절효소 활성 및 인간끝분절효소 역전사효소 발현의 조절

박육필^{1,2} · 최승철¹ · 조미영¹ · 송은영¹ · 김재화¹ · 백상기² · 김영권³ · 김종원⁴ · 이희구¹

한국생명공학연구원 세포체 연구단, 충남대학교 생물학과, 건양대학교 임상병리학과³, 단국대학교 의과대학 진단검사의학과⁴

Modulation of Telomerase Activity and Human Telomerase Reverse Transcriptase Expression by Caspases and Bcl-2 Family Proteins in Cisplatin-Induced Cell Death

Yuk Pheel Park^{1,2}, Seung-Chul Choi¹, Mi-Young Cho¹, Eun Young Song, Ph.D.¹, Jae Wha Kim, Ph.D.¹, Sang-Gi Paik, Ph.D.², Young Kwon Kim, Ph.D.³, Jong Wan Kim, M.D.⁴, and Hee Gu Lee, Ph.D.¹

Cellomics Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB)¹, Daejeon; Department of Biology, Chungnam National University², Daejeon; Department of Laboratory Medicine, Konyang University³, Nonsan; Department of Laboratory Medicine, Dankook University College of Medicine⁴, Cheonan, Korea

Background : Human telomerase is a ribonucleoprotein polymerase, which synthesizes telomeric repeat sequences, and human telomerase reverse transcriptase (hTERT) has been identified as the catalytic subunit, as well as the rate-limiting component, of telomerase. In this study, we attempted to identify the modulators of telomerase, and to determine the molecular mechanisms underlying cisplatin-induced apoptosis.

Methods : To determine the role of telomerase in cisplatin-induced apoptosis, we measured telomerase activity and analyzed apoptosis using PI and trypan blue staining. Also, we inhibited the caspase activations using Z-VAD-fmk to analyze the effects on expression of hTERT protein. Finally, we induced the transient co-expression of the *Bcl-2* and *Bak* genes in HEK293 cells, and then, the telomerase activity and expression of hTERT were evaluated.

Results : In the *Bcl-2*-overexpressing HeLa cells, telomerase activity was more enhanced, and cell death was reduced to 40-50% that of the mock controls. This finding suggests that *Bcl-2*-induced telomerase activity exerts an antiapoptotic effect in cisplatin-induced death. As caspase activation was inhibited via Z-VAD-fmk, the hTERT protein was recovered in the mock controls, but not in the *Bcl-2*-overexpressing cells. This suggests that the expression of hTERT can be regulated by caspases, but *Bcl-2* was located within the upstream pathway. Moreover, when the *Bcl-2* and *Bak* genes were co-transfected into the HEK293, both telomerase activity and hTERT protein were prominently reduced.

Conclusions : *Bcl-2*-induced telomerase activity inhibits cisplatin-induced apoptosis in HeLa cells, and can be regulated via both caspases and the interaction of *Bcl-2* and *Bak*. (*Korean J Lab Med* 2006;26:287-93)

접 수 : 2006년 3월 7일 접수번호 : KJLM1932
수정본접수 : 2006년 7월 14일
게재승인일 : 2006년 7월 18일
교 신 저 자 : 이 희 구
우 305-806 대전광역시 유성구 어은동 52
한국생명공학연구원 세포체 연구단
전화 : 042-860-4182, Fax : 042-860-4593
E-mail : hglee@kribb.re.kr

Key Words : Apoptosis, Cisplatin, hTERT, Bcl-2, Bak

INTRODUCTION

Human telomerase is a ribonucleoprotein (RNP) complex which is responsible for the elongation of telomeres,

*This study was supported by grants of FG05-40-01, NTM 0020213 of the 21C frontier function human genome project from ministry of science & technology of Korea.

thereby allowing for the maintenance of genomic integrity. Human telomerase is comprised of telomerase reverse transcriptase (hTERT), and telomerase RNA component (hTR), as well as some associated factors which regulate the catalytic activity of telomerase[1, 2]. Recent findings have revealed that telomerase confers an additional function required for tumorigenesis, which is independent of its ability to maintain the telomere[3]. Additionally, telomerase contributes to the installation of an immortal cell phenotype via the prevention of apoptosis and may also perform a significant function in cellular resistance against anticancer drugs[4-8].

In previous reports, the suppression of telomerase enzyme activity promotes apoptosis in neuronal cells[9], germ cells [10], and thymocytes[11], whereas the overexpression of hTERT prevents apoptosis via interference with a pre-mitochondrial step in the cell death cascade[12-14]. Enhancements of telomerase activity via *Bcl-2* gene overexpression have also been detected in cervical carcinoma, HeLa, and colorectal carcinoma DiFi cells[15]. However, this phenomenon, with the exception of the known possibility of a correlation between *Bcl-2* and telomerase in cancer progression. For example, in high-grade non-Hodgkin lymphoma, high expression levels of hTERT mRNA may be related to shorter survival, and telomerase activity has been shown to be affected by the expressions of *Bax* and *Bak*[16]. However, the molecular mechanisms underlying the interactions between telomerase and these regulators remain unclear, despite the extensive efforts of a number of research groups that have attempted to dissect such mechanisms.

In this study, we focused on the functions of telomerase beyond reverse transcriptase in cancer progression. In an effort to identify the modulators of telomerase, we utilized an *in vitro* cisplatin (cis-diammine-dichloro-platinum II)-induced apoptosis system.

MATERIALS AND METHODS

1. Cell culture

Human cervical cancer cell line HeLa and human embryonic kidney 293 cells were maintained in DMEM medium (GIBCO, Grand Island, NY, USA) supplemented with 10% FBS (Hyclone, Road Logan, Utah, USA) in a humidified incubator at 37°C.

2. Antibodies and reagents

Anti-*Bcl-2* monoclonal antibody (C-2) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and the anti-*Bak* monoclonal antibody was purchased from BD Biosciences (BD PharMingen, San Diego, CA, USA). Rabbit anti-hTERT polyserum was acquired from Merck Biosciences (Calbiochem, La Jolla, CA, USA), and the broad caspase inhibitor (Z-VAD-fmk) was acquired from Promega (Promega, Madison, WI, USA). All other reagents used in this study were purchased from Sigma (St. Louis, MO, USA) unless otherwise indicated.

3. Cytotoxicity Assay

To determine the role of telomerase in the programming of apoptotic death, we analyzed the cytotoxic effects of cisplatin in cervical cancer, HeLa cells. Exponentially-growing HeLa cells were treated with cisplatin, and the percentage of viable cells was determined using MTS cell proliferation reagent (Promega). The cells were plated in 96-well plates at a concentration of 1×10^4 cells/well in 100 μ L of medium. After overnight incubation at 37°C, the media were exchanged with various concentrations of cisplatin (1-20 μ g/mL). After an additional 24 hr of incubation at 37°C, 50 μ L of MTS solution was added to each wells and incubated for further 30 min at 37°C. Optical absorbance was then measured at 490 nm, using a microculture plate reader.

4. Construction of expression vectors and transfection

Wild-type *Bcl-2* cDNA was generously provided by the Laboratories of Anticancer Research (KRIBB, Daejeon, Korea), and the *Bcl-2* cDNA fragment was cloned into pcDNA3-neomycin (Invitrogen, Carlsbad, CA, USA). The pME18s-human Bak plasmid was kindly provided by the Genome Research Center (KRIBB). The fragment of human *bak* cDNA was transferred to the pCMV Tag plasmid (Invitrogen), and these constructs were verified via DNA sequence analysis. The transient transfection of HeLa or HEK293 with *Bcl-2* and/or *Bak* plasmids was conducted using Lipofectamine/Plus reagent (Invitrogen), in accordance with the manufacturer's instructions. After 24 hr of incubation, cisplatin was administered, after which the samples were incubated for an additional 24 hr.

5. Telomeric repeat amplification protocol (TRAP) assay

Telomerase activity was measured using a TRAPeze kit (Intergen, Purchase, NY, USA) in accordance with the manufacturer's recommendations. In brief, the cisplatin-treated cells were lysed in CHAPS buffer and incubated on ice for 30 min, after which the soluble proteins were obtained. The concentrations of proteins were then measured using a BioRad protein assay kit (BioRad Laboratories, Richmond, CA, USA). To allow for the quantitative assessment of the activity, 0.5 μ g of protein extracts were used in the presence of an internal TRAP assay standard (36 bp). The telomeres were extended via 30 min of incubation at 30°C, then amplified via two-step PCR (94°C for 30 sec and 60°C for 30 sec) conducted for 25–30 cycles. The radioactive TRAP products were separated on 12.5% neutral polyacrylamide gel, then autoradiographed.

6. Flow cytometry analysis of apoptosis

For the cell death analyses, the cells were harvested and washed in PBS. After 30 min of fixation with 75% ethanol at 4°C, the cells were washed with PBS three times and stained with propidium iodide (20 μ g/mL PI, 0.1 μ g/mL RNase A in PBS) in a dark room. Apoptotic cell death was then visualized via flow cytometry, using the FACS Caliber and CellQuest software packages (Becton Dickinson, Mountain View, CA, USA).

7. Trypan blue staining

The cells were stained with trypan blue (Sigma) and were counted using a hemocytometer. The percentage of cell death was expressed as the ratio of the number of trypan blue-permeable cells to the total cell count (trypan blue-permeable cell number/total cell number).

8. Western blot analysis

For Western blot analyses, the cisplatin-treated or transfected cells were lysed in a lysis buffer (20 mM Tris, pH 7.4, 2 mM EDTA, 150 mM sodium chloride, 1 mM sodium deoxycholate, 1% Triton X-100, 10% glycerol, 1 mM PMSF, 5 μ g/mL aprotinin, and 10 μ g/mL leupeptin), and the protein concentrations were determined using a BioRad protein assay kit (BioRad): 50–150 μ g of pro-

tein was subjected to 12–15% SDS-PAGE and transferred onto PVDF membranes (Hybond-P, Amersham Biosciences, Buckinghamshire, England). Subsequently, the membranes were incubated for 2 hr at room temperature in a 5% skim milk solution, then probed overnight at 4°C with the appropriate primary antibodies. The bound antibodies were then visualized using a suitable secondary antibody conjugated with horseradish peroxidase, using enhanced chemiluminescence reagents (ECL, Amersham Biosciences).

RESULTS

1. Telomerase plays an antiapoptotic role in cisplatin-induced cell death

Cisplatin inhibited the viability of HeLa cells dose-dependently (Fig. 1A), and telomerase activity was transiently increased as the consequence of cisplatin treatment, but its activity with regard to *Bcl-2* expression was enhanced to a greater degree than that of controls (Fig. 1B). Therefore, we concluded that the enhanced activity effected by the expression of *Bcl-2* also affected cisplatin-induced cell death, and the phenotype of cell death was determined via staining with propidium iodide (Fig. 1C) or trypan blue (Fig. 1D). As shown in Fig. 1C, apoptotic cell death was reduced to 40–50% in the *Bcl-2* overexpressed cells as compared to the mock controls, and the trypan blue staining data was similar to that of the PI staining data (Fig. 1D). In conclusion, telomerase activity is closely associated with cisplatin-induced cell death, and the enhanced activity affected by *Bcl-2* exerts an anti-apoptotic effect.

2. TERT expression was dependent on caspase activation in cisplatin-induced cell death

As shown in Fig. 2, about a two-fold increase in the quantity of hTERT protein was recovered as the result of Z-VAD-fmk inhibition in the mock control, but no change was detected in the *Bcl-2* overexpressed cells. This finding suggests that the expression of hTERT was dependent upon the activation of caspase, but not on *Bcl-2*, which was located within the upstream pathway.

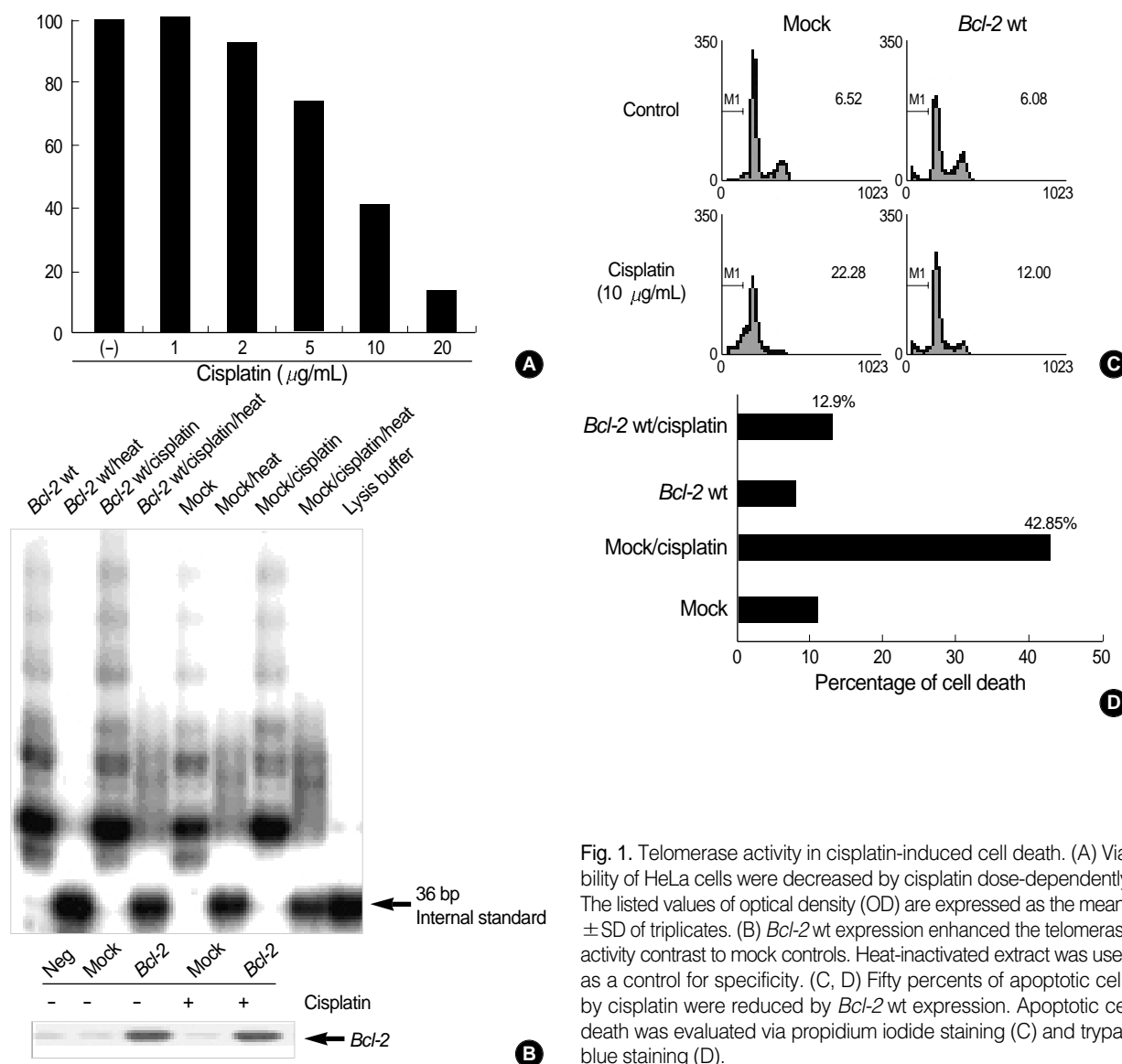


Fig. 1. Telomerase activity in cisplatin-induced cell death. (A) Viability of HeLa cells were decreased by cisplatin dose-dependently. The listed values of optical density (OD) are expressed as the means \pm SD of triplicates. (B) *Bcl-2* wt expression enhanced the telomerase activity contrast to mock controls. Heat-inactivated extract was used as a control for specificity. (C, D) Fifty percents of apoptotic cells by cisplatin were reduced by *Bcl-2* wt expression. Apoptotic cell death was evaluated via propidium iodide staining (C) and trypan blue staining (D).

3. Co-expressions of *Bcl-2* and *Bak* modulate the telomerase activity and TERT expression

In order to elucidate the interactions between wild type *Bcl-2* and pro-apoptotic *Bak* on the function of telomerase, we transiently co-transfected a pcDNA-neo plasmid encoding for wt *Bcl-2*, or a pCMV Tag-neo plasmid encoding for *Bak* or control vectors, into the HEK293 cells. As shown in Fig. 3A, telomerase activity was found to be increased slightly by *Bak* expression, just as can be seen with cisplatin treatment in Fig. 1B. However, in case *Bcl-2* and *Bak* were co-expressed at the same level, telomerase activity was reduced markedly. Evidently, hTERT protein was decreased to a greater degree on the West-

ern blots than was seen in the vector control cells. Also, *Bcl-2* protein expression was affected by *Bak* expression, but not *Bak*. The targeting of *Bcl-2* by *Bak* may have an influence on the expression of hTERT (Fig. 3B). Therefore, our findings suggest that both telomerase activity and hTERT expression may be regulated via intracellular *Bcl-2* expression, and that this can be regulated in turn by the interactions occurring between *Bcl-2* and *Bak*.

DISCUSSION

In an effort to identify the modulators of telomerase in the progression of cancers, we utilized a cisplatin-induced

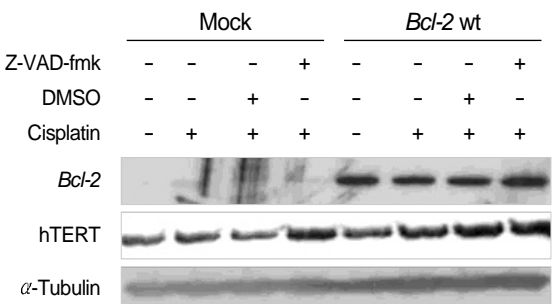


Fig. 2. hTERT expression by Z-VAD-fmk inhibition in cisplatin-induced cell death. *Bcl-2* and hTERT expression were evaluated via Western blot analysis. hTERT expression was changed by caspase inhibition. hTERT protein level was more increased by pretreatment of Z-VAD-fmk in mock control cells but not in *Bcl-2* wt expressing cells.

apoptotic program as an *in vitro* model. Since its introduction into clinical trials, cisplatin has had a significant impact in the field of cancer medicine, changing the course of therapeutic management of several cancers, including those of the ovary, testis, head, and neck[17]. However, the primary obstacles to anticancer chemotherapy include the cytotoxicity of anticancer agents to normal cells, as well as the occurrence of tumor cells that exhibit resistance to chemotherapeutic agents. In the majority of cases, the level of resistance is less than 50-fold; however, even a small increase in the cisplatin-resistance of a tumor can prove clinically important, as large dose escalations result in severe multiorgan toxicity[18]. Therefore, novel chemotherapeutic strategies, which might serve to reduce the cytotoxicity of normal cells and reverse the chemoresistance observed in tumor cells, represent an important target for the development of selective cancer therapies.

Currently, in order to inhibit telomerase, a variety of strategies have been adopted, including the introduction of TERT antisense oligomer[19, 20], G-quadruplex-interactive agents[21, 22], or small interference RNA[23]. In this study, we hypothesized that the targeting of the signaling modulators of telomerase might induce apoptosis in cancer cells. Therefore, we transiently overexpressed *Bcl-2* cDNA in HeLa cervical cancer cells. The telomerase activity and apoptotic cell death induced by *Bcl-2* were measured and evaluated (Fig. 1B-D). As shown in Fig. 1B, telomerase activity was augmented by cisplatin signals, but *Bcl-2* expression enhanced its activity prominently. This suggests that telomerase activity may be more closely associated with cisplatin-induced apoptosis, and also exert an antiapoptotic effect.

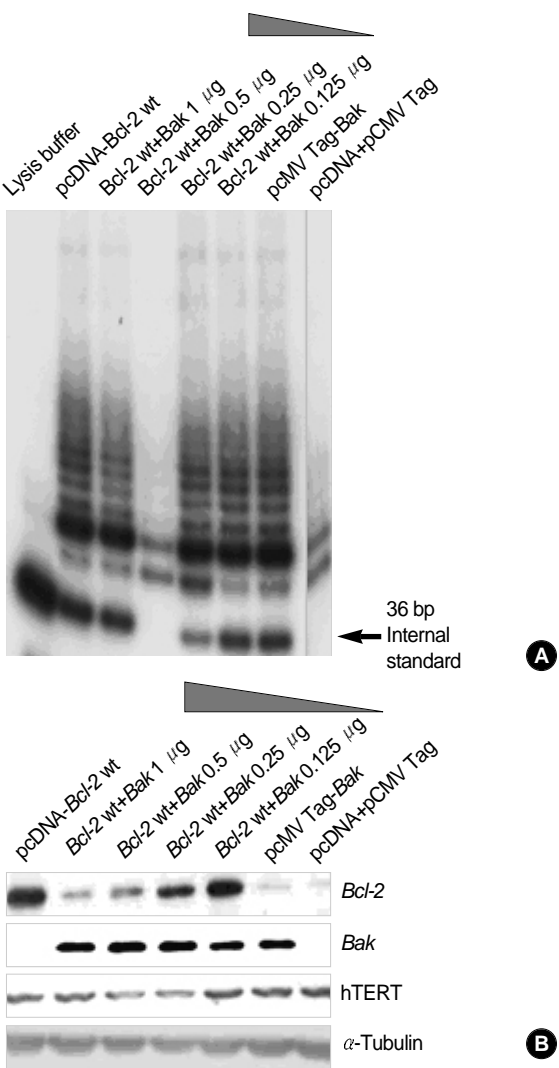


Fig. 3. Telomerase activity and hTERT expression by interaction of *Bcl-2* with *Bak*. (A) HEK293 cells were co-transfected with a *Bcl-2* wt (0.5 µg) and *Bak* (1-0.125 µg), or empty vectors. After 24 hr of incubation, telomerase activity was measured. Telomerase activity in *Bcl-2* and 0.5 µg of *Bak* co-expressing cells was reduced in contrast to other *Bcl-2* wt expressing cells. (B) Western blot analysis for the detection of *Bcl-2* and *Bak* or hTERT. Reduced expression of hTERT appeared in *Bcl-2* wt and 0.25-0.5 µg of *Bak* co-expressing cells. Alpha tubulin was utilized to ensure equal loading.

Next, to determine in which caspase proteins affect telomerase function, we inhibited caspase activations via the application of Z-VAD-fmk. The expression of hTERT, the catalytic subunit of telomerase, was recovered markedly; however, no change was observed in the *Bcl-2*-over-expressing cells (Fig. 2). Thus, we conclude that telomerase activation is located downstream of the caspases, even though *Bcl-2* existed in the upstream signal pathway, and also that the HeLa cells in our study underwent a cas-

pase-dependent cell death as the result of cisplatin administration (data not shown). Previous reports have suggested that the ratio between anti- and pro-apoptotic proteins is the primary determinant of survival or cell death[24]. Therefore, we co-transfected both *Bcl-2* and *Bak* genes into HEK293 cells, and measured both telomerase activity and hTERT expression. The co-expressions of both genes at the same ratio resulted in a reduction in the telomerase activity and hTERT expression. Indeed, the expression patterns of hTERT and *Bcl-2* were found to be quite similar (Fig. 3). These findings suggest that the antiapoptotic effects of *Bcl-2* were blocked in a reciprocal fashion by *Bak* expression. The interaction of *Bcl-2* with *Bak* can modulate both telomerase activity and TERT expression, and can then regulate the apoptotic process.

요 약

배경 : 인간 끝분절효소(telomerase)는 텔로미어(telomere) 반복서열을 합성하는 리보핵산 합성효소이며, 인간 hTERT (human telomerase reverse transcriptase)는 끝분절효소의 rate-limiting 요소일 뿐 아니라 활성단위체로서 동정되었다. 본 연구에서는 끝분절효소 조절자를 동정하고, 시스플라틴 유도 세포사멸에서의 분자적 메커니즘을 분석하고자 하였다.

방법 : 시스플라틴 유도 세포사멸에서의 끝분절효소의 기능을 규명하기 위해, 끝분절효소 활성을 측정하고, 세포사멸을 PI 염색과 trypan blue 염색으로 분석하였다. 또한, hTERT 단백질 발현에 있어서의 효과를 결정하기 위해 Z-VAD-fmk로 caspase 활성을 억제하였다. 마지막으로 HEK293 세포주에 *Bcl-2*와 *Bak* 유전자를 일시적으로 동시에 과발현시킨 다음, 끝분절효소 활성 및 hTERT 발현을 분석하였다.

결과 : *Bcl-2*를 과발현하는 HeLa 세포에서는 끝분절효소 활성이 더욱 증가되었고, 세포사멸이 mock 대조군에 비해 40-50% 정도 감소되었다. 이는 *Bcl-2*에 의해 유도된 끝분절효소활성이 시스플라틴-유도 세포사멸에서 항·세포사멸적 효과를 발휘함을 제시한다. Z-VAD-fmk로 caspase의 활성화를 억제함으로써 mock 대조군 세포에서는 hTERT 단백질 발현이 회복되나, *Bcl-2* 발현 세포에서는 변화가 없었다. 이는 hTERT의 발현이 caspase에 의해 조절될 수 있으나, *Bcl-2*는 상위 신호전달 체계에 존재하고 있음을 제시한다. 또한, HEK293 세포주에 *Bcl-2*와 *Bak* 유전자를 동시에 과발현 시켰을 때, 끝분절효소 활성과 hTERT 단백질 발현이 뚜렷이 감소되었다.

결론 : *Bcl-2* 발현에 의해 유도된 끝분절효소활성은 HeLa 세포에서의 시스플라틴-유도 세포사멸을 억제하며, 이는 caspase와 *Bcl-2*, *Bak*의 상호작용에 의해서도 조절될 수 있음을 제시한다.

ACKNOWLEDGEMENTS

We thank Dr. Lee Jeong-Hyung, at the Laboratories of Anticancer Research (KRIBB), for the wild type-*Bcl-2* plasmid, as well as Dr. Kim Nam-Soon, at the Genome Research Center (KRIBB), for the pME18s-human *Bak* plasmid.

REFERENCES

- Blackburn EH. Structure and function of telomeres. *Nature* 1991;350:569-73.
- Blackburn EH. "Switching and signaling at the telomere." *Cell* 2001;106:661-73.
- Stewart SA, Hahn WC, O'Connor BF, Banner EN, Lundberg AS, Modha P, et al. Telomerase contributes to tumorigenesis by a telomere length-independent mechanism. *Proc Natl Acad Sci USA* 2002;99:12606-11.
- Kondo Y, Kondo S, Tanaka Y, Haqqi T, Barna BP, Cowell JK. Inhibition of telomerase increases the susceptibility of human malignant glioblastoma cells to cisplatin-induced apoptosis. *Oncogene* 1998;16:2243-8.
- Fu W, Begley JG, Killen MW, Mattson MP. Anti-apoptotic role of telomerase in pheochromocytoma cells. *J Biol Chem* 1999;274:7264-71.
- Fu W, Killen M, Culmsee C, Dhar S, Pandita TK, Mattson MP. The catalytic subunit of telomerase is expressed in developing brain neurons and serves a cell survival-promoting function. *J Mol Neurosci* 2000;14:3-15.
- Kushner DM, Paranjape JM, Bandyopadhyay B, Cramer H, Leaman DW, Kennedy AW, et al. 2-5A antisense directed against telomerase RNA produces apoptosis in ovarian cancer cells. *Gynecol Oncol* 2000;76:183-92.
- Dudognon C, Pendino F, Hillion J, Saumet A, Lanotte M, Segal-Bendirdjian E. Death receptor signaling regulatory function for telomerase: hTERT abolishes TRAIL-induced apoptosis, independently of telomere maintenance. *Oncogene* 2004;23:7469-74.
- Mattson MP and Klapper W. Emerging roles for telomerase in neuronal development and apoptosis. *J Neurosci Res* 2001;63:1-9.
- Hemann MT, Rudolph KL, Strong MA, DePinho RA, Chin L, Greider CW. Telomere dysfunction triggers developmentally regulated germ cell apoptosis. *Mol Biol Cell* 2001;12:2023-30.
- Ichiyoshi H, Kiyozuka Y, Kishimoto Y, Fukuhara S, Tsubura A. Massive telomere loss and telomerase RNA expression in dexamethasone-induced apoptosis in mouse thymocytes. *Exp Mol Pathol* 2003;

- 75:178-86.
12. Zhang P, Chan SL, Fu W, Mendoza M, Mattson MP. TERT suppresses apoptosis at a premitochondrial step by a mechanism requiring reverse transcriptase activity and 14-3-3 protein-binding ability. *FASEB J* 2003;17:767-9.
 13. Gorbunova V, Seluanov A, Pereira-Smith OM. Expression of human telomerase (hTERT) does not prevent stress-induced senescence in normal human fibroblasts but protects the cells from stress-induced apoptosis and necrosis. *J Biol Chem* 2002;277:38540-9.
 14. Holt SE, Glinsky VV, Ivanova AB, Glinsky GV. Resistance to apoptosis in human cells conferred by telomerase function and telomere stability. *Mol Carcinogen* 1999;25:241-8.
 15. Mandal M and Kumar R. *Bcl-2* modulates telomerase activity. *J Biol Chem* 1997;272:14183-7.
 16. MacNamara B, Wang W, Chen Z, Hou M, Mazur J, Gruber A, et al. Telomerase activity in relation to pro- and anti-apoptotic protein expression in high grade non-Hodgkin's lymphomas. *Haematologica* 2001;86:386-93.
 17. Loehrer PJ and Einhorn LH. Drugs five years later. Cisplatin. *Ann Intern Med* 1984;100:704-13.
 18. Chu G, Mantin R, Shen YM, Baskett G, Sussman H. Massive cisplatin overdose by accidental substitution for carboplatin. Toxicity and management. *Cancer* 1993;72:3707-14.
 19. Folini M, Brambilla C, Villa R, Gandellini P, Vignati S, Paduano F, et al. Antisense oligonucleotide-mediated inhibition of hTERT, but not hTERC, induces rapid cell growth decline and apoptosis in the absence of telomere shortening in human prostate cancer cells. *Eur J Cancer* 2005;41:624-34.
 20. Kraemer K, Fuessel S, Schmidt U, Kotzsch M, Schwenzer B, Wirth MP, et al. Antisense-mediated hTERT inhibition specifically reduces the growth of human bladder cancer cells. *Clin Cancer Res* 2003;9:3794-800.
 21. Tahara H, Shin-Ya K, Seimiya H, Yamada H, Tsuruo T, Ide T. G-Quadruplex stabilization by telomestatin induces TRF2 protein dissociation from telomeres and anaphase bridge formation accompanied by loss of the 3' telomeric overhang in cancer cells. *Oncogene* 2006;25:1955-66.
 22. Tauchi T, Shin-Ya K, Sashida G, Sumi M, Okabe S, Ohyashiki JH, et al. Telomerase inhibition with a novel G-quadruplex-interactive agent, telomestatin: in vitro and in vivo studies in acute leukemia. *Oncogene* 2006 in press.
 23. Pallini R, Sorrentino A, Pierconti F, Maggiano N, Faggi R, Montano N, et al. Telomerase inhibition by stable RNA interference impairs tumor growth and angiogenesis in glioblastoma xenografts. *Int J Cancer* 2006;118:2158-67.
 24. Yin XM, Oltvai ZN, Korsmeyer SJ. BH1 and BH2 domains of *Bcl-2* are required for inhibition of apoptosis and heterodimerization with *Bax*. *Nature* 1994;369:321-3.