J. Life Science. (2000), 10(2), 39~44

JOURNAL OF
Life Science

Trichostatin A Induces Apoptotic Cell Death in Human Breast Carcinoma Cells through Activation of Caspase-3

Seaho Kim, Yung Hyun Choi*, Eun Ok Im, Ji Hyeon Lee, Dong-Kyoo Kim and Nam Deuk Kim†**

Department of Pharmacy, Pusan National University and Pusan Cancer Research Center, Pusan 609-735
**Department of Biochemistry, College of Oriental Medicine, Dong-Eui University and Research Center for Oriental Medicine, Pusan 614-052*
***Department of Chemistry, Inje University, Kimhae 621-170, Korea*

Received: October 14, 2000

Abstract Trichostatin A (TSA) is a *Streptomyces* product, which inhibits the enzyme activity of histone deacetylase. It is also known as an inducer of apoptosis on several human cancer cell lines. In this study, we investigated the mechanism of apoptosis induced by TSA in MDA-MB-231 human breast carcinoma cells. The cytotoxicity of TSA on MDA-MB-231 cells was assessed by MTT assay. The cell viability was decreased dose-dependently and the IC_{50} value was about 100 ng/ml after 48 h treatment with TSA. Morphological change and DNA ladder formation, the biochemical hallmarks of apoptotic cell death, were observed after treatment of TSA in a concentration-dependent manner, which was accompanied with cleavage of poly(ADP-ribose) polymerase and β-catenin, and activation of caspase-3. TSA treatment up-regulated the expression of a cyclin-dependent kinase inhibitor p21 (Waf1/Cip1) protein, a key regulatory protein of the cell cycle. However, there is no detectable change of both Bcl-2 and Bax expressions. These results demonstrated that TSA might inhibit cell growth through apoptosis in human breast carcinoma MDA-MB-231 cells.

Key words: Trichostatin A, Apoptosis, Caspase-3, p21

Introduction

Apoptosis, a normal mechanism for deleting unwanted or moderately damaged cells, has important implications for cancer; suppression of apoptosis promotes tumor growth and, many anticancer agents induce it [1,2]. During induction of apoptotic cell death, proteolysis plays a crucial role and several members of the interleukin-1β-converting enzyme (ICE) family that can induce apoptosis when activated or over expressed in various cell types have been identified [3-5]. Several caspase substrates have been identified and include nuclear proteins such as poly(ADP-ribose) polymerase (PARP) and retinoblastoma protein (pRB) as well as structural proteins of the nucleus and cytoskeleton including lamins and β-catenin [6,7]. It is believed that cleavage of some of the known caspase substrates leads to the characteristic changes in morphology and biochemistry observed in apoptotic cells [8]. As an example, caspase-3 mediated cleavage of DNA fragmentation factor results in chromatin condensation and DNA fragmentation during apoptosis [9,10].

Trichostatin A (TSA) is a *Streptomyces* product which causes the induction of cell differentiation and specific inhibition of the cell cycle in the G1 and G2 phases at the very low concentration [11,12]. It has been reported that TSA activates caspase-3 and induces apoptosis in a certain cancer cells including Jurkat lymphoid and LIM 1215 colorectal cancer cells [13,14]. In addition, the induction of cyclin-dependent kinase (CdK) inhibitor p21 and cell growth arrest caused by TSA in p53-depicient Hep3B cells [15]. Even though cell cycle alteration caused by TSA was confirmed [16], there is no study about the involvement of apoptotic executioner mediated by TSA in MDA-MB-231 human breast carcinoma cells. The purpose of the present study was to determine how TSA mediated apoptosis in MDA- MB-231 cells. Our results indicate that TSA-mediated apoptosis is not associated with changes in the levels of Bcl-2 and Bax expression. However, caspase-3 was proteolytically activated and Cdk inhibitor p21 was up-regulated in response to TSA.

Materials and Methods

Cell Culture and Trichostatin A

The human mammary gland adenocarcinoma cell line MDA-MB-231 was obtained from ATCC (American Tissue Culture Collection, Rockville, MD, USA). Cells were grown

*Corresponding author
Phone: 82-51-510-2801, Fax: 82-51-513-6754
E-mail: nadkim@hyowon.pusan.ac.kr

in DMEM (Dulbecco's Modified Eagle's medium) with 10% fetal bovine serum in a humidified 5% CO_2 at 37℃ containing 50 mg/ml gentamicin, 135 mg/ml glutamine. TSA (Fig. 1) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The drug was dissolved as a stock solution in dimetyl sulfoxide (DMSO) at a concentration of 1 mg/ml, and aliquots were stored at -20℃.

MTT Assay

Cell survival was quantified by colorimetric MTT assay, which measures mitochondrial activity in viable cells. For this assay, cells seeded at a density of 6 x 10^4/well in 24 wells plate, were allowed to adhere overnight and then the culture medium was replaced with fresh medium. Cells were exposed to TSA and after 48 h the medium was replaced with serum free medium. 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrasolium bromide (MTT, Sigma) was freshly prepared at 5 mg/ml in phosphate-buffered saline (PBS) and passed through a 0.2-pore-size filter. An aliquot of 100 μl of MTT stock solution was added to each well, and the plate was incubated for 4 h at 37℃. After 4 h incubation medium were removed. To each well, 1 ml of ethanol-DMSO (1:1 mixture solution) was added in order to dissolve the formazan, which was measured after 10 min. The optical density of each well was measured with a spectrophotometer equipped with a 540-nm filter. Proliferation rate was calculated from 4 wells using percentage of control.

DNA Purification and Agarose Gel Electrophoresis

Cells were rinsed twice in cold PBS. Genomic DNA was extracted from 1 x 10^6 cells as described [17]. Briefly, cells were resuspended in a lysis buffer (5 mM Tris-Cl, pH 7.4, 20 mM EDTA, and 0.5% Triton X-100) for 30 min. The supernatants from the lysate were treated with RNase, followed by proteinase K digestion, phenol chloroform extraction and isopropanol precipitation. DNA was separated through 1.5% agarose gel. After electrophoresis, gel was stained with ethidium bromide (EtBr, Sigma) and visualized by UV light.

Protein Preparation and Western Immunoblotting

Cells were harvested and lysed in lysis buffer (40 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.5% NP-40, 0.1 mM sodium orthovanadate, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 100 μg/ml PMSF). Protein concentration was then measured using protein assay reagents (Pierce, IL, USA). Cell extracts were boiled for 3 min and chilled on ice, subjected to SDS-polyacrylamide gels, and electrophoretically transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH, USA). Western blot analysis was performed as described [18]. Monoclonal antibodies to p53, p21, Bcl-2, and β-catenin, and polyclonal antibodies to Bax, caspase-3, PARP were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Monoclonal anti-β-actin antibody was obtained from Sigma. Peroxidase-labeled donkey anti-rabbit immunoglobulin and peroxidase-labeled sheep anti-mouse immunoglobulin, and enhanced chemiluminescence (ECL) kit were purchased from Amersham Corp. (Arlington Heights, IL, USA).

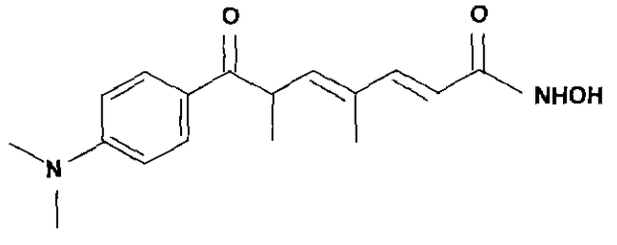

Fig. 1. Chemical structure of Trichostatin A (TSA).

Results

Cytotoxic Effect and Morphological Changes by TSA Treatment

The cytotoxicity of TSA on MDA-MB-231 cells was measured by MTT assay (Fig. 2A). After 48 h treatment of cells with various concentrations of TAS, the viability was mark-

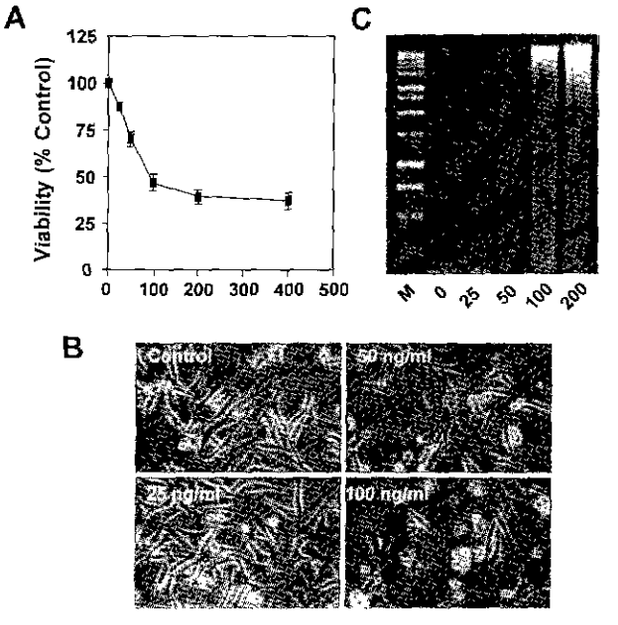

Fig. 2. TSA-induced anti-proliferative effect was associated with morphological changes and DNA fragmentation in human breast carcinoma MDA-MB-231 cells. Exponentially growing cells were incubated with either vehicle (DMSO) alone or indicated concentrations (ng/ml) of TSA for 48 h. (A) The cell viability was measured by MTT assay after 48 h treatment with indicated concentrations (ng/ml) of TSA. Results are means±S.D. from three separate experiments. (B) Cell morphology was visualized by light microscopy. Magnification, X200. M, 100-bp ladder was used as marker. (C) Total genomic DNA was extracted and resolved in agarose gel and visualized using EtBr.

edly decreased in a dose-dependent manner and the IC_{50} value was about 100 ng/ml. This anti-proliferative effect of TSA was accompanied by membrane shrinking and cell rounding up (Fig. 2B). These distinct morphological changes were even more pronounced with higher concentration of TSA.

Induction of Apoptosis by TSA

We next determined whether TSA induces apoptotic cell death in MDA-MB-231 cell. For this purpose, agarose gel electrophoresis and Western immunoblotting experiments were conducted. As shown in Fig. 4, treatment of cells with TSA resulted in a concentration-dependent increase in DNA fragmentation, which is a hallmark of apoptosis. Result of Western blot analysis for PARP expression showed that the PARP proteins were proteolytically cleavaged, with the concomitant diminution of full size (116 kDa) molecule and accumulation of the 89 kDa, in TSA treated-cells (Fig. 3). The degradation of β-catenin was also observed by Western blot analysis. In control cells, β-catenin was detected as a 92 kDa proteins, however, the cleavage fragments in TSA-treated cells were not detectable (Fig. 3). These results indicated that the anti-proliferative effect of TSA was associated with apoptosis.

Activation of Caspase-3

Caspases are believed to play a central role in the apoptotic signaling pathway. Among them, caspase-3 normally exists as an inactive proenzyme (about 32 kDa) but is cleaved to a heterodimer of 19 and 17 kDa subunits that constitute the active protease just immediately prior to apoptotic cell death [19]. Activation of caspases leads to the cleavage

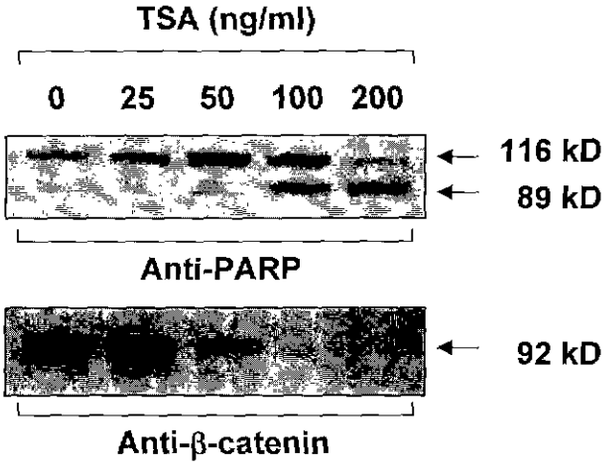

Fig. 3. Degradation of PARP and β-catenin proteins during TSA-mediated apoptosis in MDA-MB-231 cells. Cells were treated with TSA for 48 h, lysed and then equal proteins were resolved on SDS-polyacrylamide gels. Proteins were visualized by Western blot analysis using anti-PARP and anti-β-catenin antibodies, and ECL detection kit.

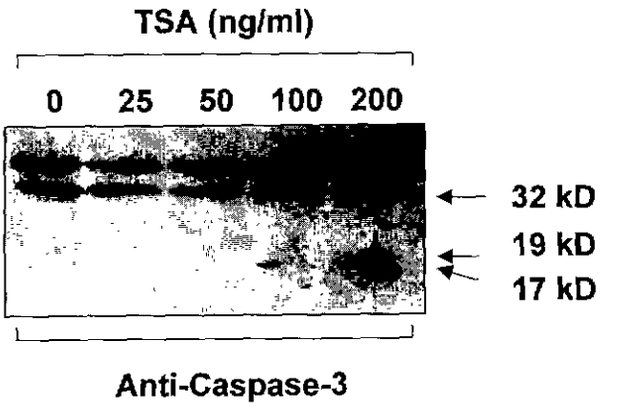

Fig. 4. Effect of TSA on the expression of caspase-3 in MDA-MB-231 cells. After treatment with TSA for 48 h, total cell lysates were prepared and equal amounts of proteins were separated by SDS-polyacrylamide gel, transfer to nitrocellulose membrane. Western immunoblotting was detected with anti-caspase-3 antibody and ECL detection.

of target proteins including PARP and β-catenin, which are also hallmarks of apoptosis and substrates of activated caspases. As illustrated in Fig. 4, treatment of cells with TSA caused a dose-dependent cleavage of caspase-3 to yield 19 and 17 kDa fragments, which is in good agreement to a proteolytic cleavage of PARP and β-catenin proteins by TSA.

Effects of TSA in Bcl-2 and Bax expression

It is well known that the ratio of anti- to pro-apoptotic molecules such as Bcl-2 and Bax determines the response to death signals, which cause mitochondrial dysfunction and release of cytochrome c into cytosol, followed by induction and/or activation of p53, caspases and endonucleases [20,21]. We, therefore, examined the potential involvement of both proteins. After exposure to different concentrations of TSA for 48 h, immunoblotting was performed using total cellular proteins. The Western blot results indicated that the levels of both Bcl-2 and Bax protein remained unchanged in TSA-treated cells (Fig. 5), which suggested that TSA did not affect the alteration of the balance between Bcl-2 and Bax expression in MDA-MB-231 cells.

Induction of Cdk Inhibitor p21

Because Cdk inhibitor p21 is universal regulator in both growth arrest and apoptosis, which impose by the tumor suppressor p53 in response to DNA damage [22,23], we finally examined the effects of TSA in the levels of p53 and p21 expression. As shown in Fig. 6, the level of p53 proteins was slightly increased at 50 ng/ml concentration of TSA and then decreased at 100 and 200 ng/ml concentration. The level of p21 proteins increased until 50 ng/ml concentration of TSA but under high concentration treatment (above 100 ng/ml) the levels were decreased. The

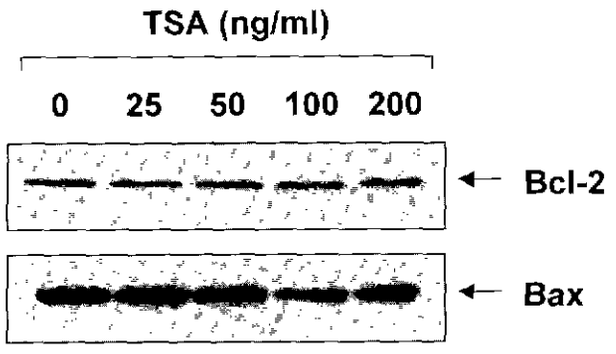

Fig. 5. Analysis of Bcl-2 and Bax protein levels in TSA-treated MDA-MB-231 cells. Cells were incubated with indicated concentrations of TSA for 48 h. Thereafter, cells were lysed and equal amounts of cellular proteins were separated by SDS-polyacrylamide gels, transfer to nitrocellulose membranes. The membranes were probed with the antibodies against Bcl-2 and Bax, respectively. Proteins were visualized using ECL detection.

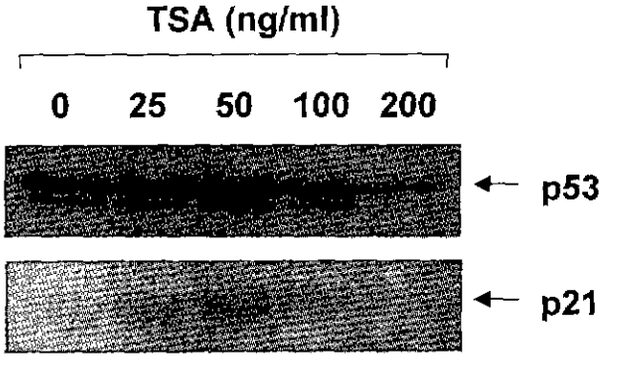

Fig. 6. Effects of TSA on the expression of tumor suppressor p53 and Cdk inhibitor p21 in MDA-MB-231 cells. After treatment with TSA for 48 h, total cell lysates were prepared and equal amounts of proteins were separated by SDS-polyacrylamide gels, transfer to nitrocellulose membranes. Western immunoblotting was detected with anti-p53 and anti-p21 antibodies, and ECL detection.

MDA-MB-231 cells have mutant form of p53. So this p21 regulation by TSA seemed to p53 independent.

Discussion

Although multiple induction pathways lead to apoptosis, apoptosis is characterized in almost all cells by similar morphological features, suggesting that the final events in the execution phase of cell death may be shared [24,25]. Whereas the molecular mechanisms that mediate these characteristic intracellular events during apoptosis are only partially characterized, it is generally accepted that activation of proteolytic cascades is involved in the execution or the regulation of apoptosis [26,27]. Caspases are synthesized as inactive proenzymes (~30-55 kDa) that are processed in cells undergoing apoptosis by self-proteolysis and cleavage by another protease. The processed forms consist of large (17~37 kDa) and/or small (10~12 kDa) subunits, which associate to form an active enzyme [28,29]. In this study, we investigated the mechanisms of TSA-induced apoptosis in human breast carcinoma MDA-MB-231 cells. As shown in Fig. 4, the treatment of TSA in MDA-MB-231 cells induced the cleavage of caspase-3. At concentration of 100 ng/ml the cleaved heterodimers of 19 and 17 kDa subunits were detected and the cleavage level of proenzyme was increased at 200 ng/ml of TSA treatment, which data supported that activation of caspase-3 is involved in TSA-mediated apoptosis in MDA-MB-231 cells. The DNA repair enzyme PARP is cleaved during apoptosis induced by variety of stimuli [6,30,31]. There are strong suggestions that the caspase (s) responsible for PARP proteolysis is also a key component of the apoptotic cell death machinery [30,32]. In this point our result of cleavage of PARP by treatment of TSA is one of major evidences of activation of caspase-3 and concomitant execution of apoptosis. As shown in Fig. 3, the PARP proteins of 116 kDa were cleaved at the concentration of 100 ng/ml TSA. And the cleaved subunits of 89 kDa were increased in a dose-dependent fashion. These points of concentration were well agreed to the activation of caspase-3. In addition, β-catenin is also reported as one of cellular target substrate of caspase-3 [33,34]. As depicted in Fig. 3, β-catenin proteins were degraded in a concentration-dependent manner in TSA-treated cells. Even though the cleavage fragments of β-catenin were not detected, a 92 kDa proteins were degraded in the same point of caspase-3 activation. Since β-catenin is implicated in cell adhesion and signal transduction, the degradation of this protein may suggest various possible associations in the dramatic cytoskeletal and morphological changes that accompany apoptosis [33].

Fragmentation of cellular DNA at the internucleosomal linker regions has been observed in cells undergoing apoptosis, which was induced by a variety of agents. This cleavage produces ladders of DNA fragments that are the sizes of integer multiples of a nucleosome length (180-200 bp) [35-37]. Recently, it has been reported that caspase-3 activates the caspase-activated DNase (CAD) responsible for DNA fragmentation by specifically cleaving and inactivating ICAD, the inhibitor of CAD [38,39]. As shown in Fig. 2, the ladder of genomic DNA was observed in the TSA-treated MDA-MB-231 cells. Accumulation of DNA fragmentation was started at 100 ng/ml concentration of TSA. This point seems to co-ordinate with activation of caspase-3 and cleavage of PARP and β-catenin. Therefore, we concluded that the activation of caspase-3 was mediated with DNA fragmentation in TSA-treated MDA-MB-231 cells. Both tumor suppressor p53 and Cdk inhibitor p21 are known to be involved in not only growth arrest but also apoptotic cell death. Additionally, functional p53 can down-regulate Bcl-2,

which allows cells to survive a variety of fatal cellular events and protects cells from apoptosis [40]. Functional p53 can also induce p21, and an increased level of p21 can decrease the activity of Cdks, resulting in growth arrest [41-44], meaning that functional p53 is important in p53-dependent pathway leading to apoptosis. The treatment of these cells with TSA up-regulated the expression of the dysfunctional p53 and p21 after treatment for 48 h at 50 ng/ml of TSA. However, both levels were decreased at 100 and 200 ng/ml of TSA (Fig. 6). MDA-MB-231 breast cancer cells harbor mutant p53. Thus, the up-regulation of p21 is resulted from p53-independent pathway and may be another molecular mechanism through which TSA inhibits cell growth and induces apoptosis.

Bcl-2 family gene products have been reported to play a major role in determining whether cells will undergo apoptosis under experimental conditions that promote cell death (45). Increased expression of Bax can induce apoptosis [40], while Bcl-2 protects cells from apoptosis. It has been suggested that that the ratio of Bax to Bcl-2, rather than Bcl-2 alone, is important for the survival of drug-induced apoptosis in various cancer cell lines. However, our data showed no significant changes of both Bcl-2 and Bax expression after treatment with TSA (Fig. 5). Thus, TSA-mediated apoptosis in MDA-MB-231 cells seemed not to associate with Bcl-2 and Bax gene products.

In conclusion, it seemed that TSA mediated activation of caspase-3 and then the activated caspase-3 executed apoptosis in human breast carcinoma MDA-MB-231 cells. This report brings a small step to understand the mechanism of apoptosis mediated by TSA. Moreover, this finding cannot explain the early interactions of TSA with cells. So additional studies of early event of TSA in this cell line are needed.

Acknowledgment

This study was supported by a grant (#HMP-98-D-4-0036) of the Good Health R&D Project, Ministry of Health & Welfare, R.O.K.

References

1. Eastman, A. 1990. Activation of programmed cell death by anticancer agents: cisplatin as a model system. *Cancer Cells*, **2**, 275-280.
2. Wyllie, A.H. 1992. Apoptosis and the regulation of cell numbers in normal and neoplastic tissues: an overview. *Cancer Metastasis Rev.*, **11**, 95-103.
3. Fernandes-Alnemri, T., G. Litwack, and E.S. Alnemri. 1994. CPP32, a novel human apoptotic protein with homology to *Caenorhabditis elegans* cell death protein Ced-3 and mammalian interleukin-1 β-converting enzyme. *J. Biol. Chem.*, **269**, 30761-30764.
4. Kumar, S., M. Kinoshita, M. Noda, N.G. Copeland, and N.A. Jenkins. 1994. Induction of apoptosis by the mouse Nedd2 gene, which encodes a protein similar to the product of the *Caenorhabditis elegans* cell death gene ced-3 and the mammalian IL-1 β-converting enzyme. *Genes Dev.*, **8**, 1613-1626.
5. Fernandes-Alnemri, T., G. Litwack, and E.S. Alnemri. 1995. Mch2, a new member of the apoptotic Ced-3/Ice cysteine protease gene family. *Cancer Res.*, **55**, 2737-2742.
6. Kaufmann, S.H., S. Desnoyers, Y. Ottaviano, N.E. Davidson, and G.G. Poirier. 1993. Specific proteolytic cleavage of poly (ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. *Cancer Res.*, **53**, 3976-3985.
7. An, B., J.R. Jin, P. Lin, and Q.P. Dou. 1996. Failure to activate interleukin 1 β-converting enzyme-like proteases and to cleave retinoblastoma protein in drug-resistant cells. *FEBS Lett.*, **399**, 158-162.
8. Cryns, V., and J. Yuan. 1998. Proteases to die for. *Genes Dev.*, **12**, 1551-1570.
9. Liu, X., H. Zou, C. Slaughter, and X. Wang. 1997. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell*, **89**, 175-184.
10. Liu, X., P. Li, P. Widlak, H. Zou, X. Luo, W.T. Garrard, and X. Wang. 1998. The 40-kDa subunit of DNA fragmentation factor induces DNA fragmentation and chromatin condensation during apoptosis. *Proc. Natl. Acad. Sci. USA*, **95**, 8461-8466.
11. Yoshida, M., Y. Hoshikawa, K. Koseki, K. Mori, and T. Beppu. 1990. Structural specificity for biological activity of trichostatin A, a specific inhibitor of mammalian cell cycle with potent differentiation-inducing activity in Friend leukemia cells. *J. Antibiot. (Tokyo)*, **43**, 1101-1106.
12. Yoshida, M., M. Kijima, M. Akita, and Beppu T. 1990. Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. *J. Biol. Chem.*, **265**, 17174-17179.
13. Medina, V., B. Edmonds, G.P. Young, R. James, S. Appleton, and P.D. Zalewski. 1997. Induction of caspase-3 protease activity and apoptosis by butyrate and trichostatin A (inhibitors of histone deacetylase): dependence on protein synthesis and synergy with a mitochondrial/cytochrome *c*-dependent pathway. *Cancer Res.*, **57**, 3697-3707.
14. Lee, E., T. Furukubo, T. Miyabe, A. Yamauchi, and K. Kariya. 1996. Involvement of histone hyperacetylation in triggering DNA fragmentation of rat thymocytes undergoing apoptosis. *FEBS Lett.*, **395**, 183-187.
15. Gray, S.G., T. Yakovleva, W. Hartmann, M. Tally, G. Bakalkin, and T.J. Ekstrom. 1999. IGF-II enhances trichostatin A-induced TGF β1 and p21(Waf1, Cip1, sdi1) expression in Hep3B cells. *Exp. Cell Res.*, **253**, 618-628.
16. Mielnicki, L.M., A.M. Ying, K.L. Head, H.L. Asch, and B.B. Asch. 1999. Epigenetic regulation of gelsolin expression in human breast cancer cells. *Exp. Cell Res.*, **249**, 161-176.
17. Choi, Y.H., J.H. Baek, M.A. Yoo, H. Chung, N.D. Kim, and K.W. Kim. 2000. Induction of apoptosis by ursolic acid through activation of caspases and down-regulation of c-IAPs in human prostate epithelial cells. *Int. J. Oncol.*, **17**, 565-571.
18. Choi, Y.H., W.H. Lee, K.Y. Park, and L. Zhang. 2000. p53-independent induction of p21 (WAF1/CIP1), reduction of cyclin B1 and G2/M arrest by the isoflavone genistein in human prostate carcinoma cells. *Jpn. J. Cancer. Res.*, **91**,

164-173.
19. Liu, X., C.N. Kim, J. Pohl, and X. Wang. 1996. Purification and characterization of an interleukin-1beta-converting enzyme family protease that activates cysteine protease p32 (CPP32). *J. Biol. Chem.*, **271**, 13371-13376.
20. Skulachev, V.P. 1998. Cytochrome c in the apoptotic and antioxidant cascades. *FEBS Lett.*, **423**, 275-280.
21. Halestrap, A.P., E. Doran, J.P. Gillespie, and A. O'Toole. 2000. Mitochondria and cell death. *Biochem. Soc. Trans.*, **28**, 170-177.
22. Gartel, A.L., M.S. Serfas, and A.L. Tyner. 1996. p21-negative regulator of the cell cycle. *Proc. Soc. Exp. Biol. Med.*, **213**, 138-149.
23. Boulaire, J., A. Fotedar, and R. Fotedar. 2000. The functions of the cdk-cyclin kinase inhibitor p21WAF1. *Pathol. Biol. (Paris)*, **48**, 190-202.
24. Eastman, A. 1993. Apoptosis: a product of programmed and unprogrammed cell death. *Toxicol. Appl. Pharmacol.*, **121**, 160-164.
25. Chinnaiyan, A.M., and V.M. Dixit. 1996. The cell-death machine. *Curr. Biol.*, **6**, 555-562.
26. Martin, S.J., and D.R. Green. 1995. Protease activation during apoptosis: death by a thousand cuts? *Cell*, **82**, 349-352.
27. Fraser, A., and G. Evan. 1996. A license to kill. *Cell*, **85**, 781-784.
28. Margolin, N., S.A. Raybuck, K.P. Wilson, W. Chen, T. Fox, Y. Gu, and D.J. Livingston. 1997. Substrate and inhibitor specificity of interleukin-1 β-converting enzyme and related caspases. *J. Biol. Chem.*, **272**, 7223-7228.
29. Alnemri, E.S., D.J. Livingston, D.W. Nicholson, G. Salvesen, N.A. Thornberry, W.W. Wong, and J. Yuan. 1996. Human ICE/CED-3 protease nomenclature. *Cell*, **87**, 171.
30. Lazebnik, Y.A., S. Cole, C.A, Cooke, W.G. Nelson, and W.C. Earnshaw. 1993. Nuclear events of apoptosis *in vitro* in cell-free mitotic extracts: a model system for analysis of the active phase of apoptosis. *J. Cell. Biol.*, **123**, 7-22.
31. Tewari, M., and V.M. Dixit. 1995. Fas- and tumor necrosis factor-induced apoptosis is inhibited by the poxvirus crmA gene product. *J. Biol. Chem.*, **270**, 3255-3260.
32. Lazebnik, Y.A., S.H. Kaufmann, S. Desnoyers, G.G. Poirier, and W.C. Earnshaw. 1994. Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature*, **371**, 346-347.
33. Fukuda, K. 1999. Apoptosis-associated cleavage of β-catenin in human colon cancer and rat hepatoma cells. *Int. J. Biochem. Cell. Biol.*, **31**, 519-529.
34. Steinhusen, U., V. Badock, A. Bauer, J. Behrens, B. Wittman-Liebold, B. Dorken, and K. Bommert. 2000. Apoptosis-induced cleavage of beta-catenin by caspase-3 results in proteolytic fragments with reduced transactivation potential. *J. Biol. Chem.*, **275**, 16345-16353.
35. Chiarugi, V., L. Magnelli, A. Turchetti, M. Cinelli, S. Cavari, and M Ruggiero. 1994. Cell survival and death programmes. *Pharmacol. Res.*, **29**, 101-110.
36. Huang, P., K. Ballal, and W. Plunkett. 1997. Biochemical characterization of the protein activity responsible for high molecular weight DNA fragmentation during drug-induced apoptosis. *Cancer Res.*, **57**, 3407-3414.
37. Fisher, D E. 1994. Apoptosis in cancer therapy: crossing the threshold. *Cell*, **78**, 539-542.
38. Sakahira, H., M. Enari, and S. Nagata. 1998. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. *Nature*, **391**, 96-99.
39. Xerri. L., F. Palmerini, E. Devilard, T. Defrance, R. Bouabdallah, J. Hassoun, and F. Birg. 2000. Frequent nuclear localization of ICAD and cytoplasmic co-expression of caspase-8 and caspase-3 in human lymphomas. *J. Pathol.*, **192**, 194-202.
40. Sano, H , Y. Kawahito, R.L. Wilder, A. Hashiramoto, S. Mukai, K. Asai, S. Kimura, H. Kato, M. Kondo, and T. Hla. 1995. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res.*, **55**, 3785-3789.
41. Vogelstein, B., and K.W. Kinzler. 1992. p53 function and dysfunction. *Cell*, **70**, 523-526.
42. El-Deiry, W.S., T. Tokino, V.E. Velculescu, D.B. Levy, R. Parsons, J.M. Trent, D. Lin, W.E. Mercer, K.W. Kinzler, and B. Vogelstein. 1993. WAF1, a potential mediator of p53 tumor suppression. *Cell*, **75**, 817-825.
43. Harper, J.W., G.R. Adami, N. Wei, K. Keyomarsi, and S.J. Elledge. 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell*, **75**, 805-816.
44. Xiong, Y., G.J. Hannon, H. Zhang, D. Casso, R. Kobayashi, and D. Beach. 1993. p21 is a universal inhibitor of cyclin kinases. *Nature*, **366**, 701-704.
45. Li, Y., S. Upadhyay, M. Bhuiyan, and F.H. Sarkar. 1999. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. *Oncogene*, **18**, 3166-3172.