Jpn. J. Cancer Res. **90**, 180–187, February 1999

Activation of Caspases in *p53*-induced Transactivation-independent Apoptosis

Chongfeng Gao and Nobuo Tsuchida[1]

Department of Molecular Cellular Oncology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549

Though *p53*-induced apoptosis plays an important role in tumor suppression, the mechanism(s) by which *p53* induces apoptosis is still unclear. To elucidate the *p53*-induced apoptotic pathway, we examined the role of *p53* transactivation activity and caspase in J138V5C cells carrying a human temperature-sensitive (*ts*) *p53* mutant (138Ala→Val). The results showed that *p53*-induced apoptosis was not blocked by cycloheximide, which effectively prevented the expression of *p53* target genes, indicating that transactivation was not essential for *p53*-induced apoptosis in this system. Western blot analysis showed that PARP, CPP32 and ICH-1 precursors were cleaved during apoptosis. The CPP32-preferential tetrapeptide inhibitor Ac-DEVD-CHO blocked the cleavage of ICH-1 and PARP precursors, suggesting that CPP32 or some other DEVD-sensitive caspase(s) is the upstream activator of ICH-1. We also examined the role of the Fas pathway by using Fas and Fas ligand-neutralizing antibodies. Both antibodies failed to block *p53*-induced apoptosis, suggesting that the Fas pathway was not essential for *p53*-induced apoptosis in this system. Taken together, our results indicate that *p53*-induced, transactivation-independent apoptosis in Jurkat cells involves sequential activation of CPP32 or some other DEVD-sensitive caspase(s) and ICH-1, via a Fas-independent pathway.

Key words: *p53* — Apoptosis — Caspase — Fas — Jurkat

Loss of *p53* function is the most frequent event in tumorigenesis.[1)] Acting as a gatekeeper, *p53* contributes to tumor suppression by sensing DNA damage and inducing cell cycle arrest or apoptosis.[2, 3)] It is well established that *p53* causes cell cycle arrest, at least in part, by transactivating $p21^{waf1/sdi1}$.[4–6)] However, the mechanism(s) by which *p53* induces apoptosis is less clear. In some systems, *p53* induces apoptosis by sequence-specifically transactivating the proapoptotic genes, including *bax* or *IGF-BP3*.[7–10)] *p53* also induces apoptosis in the absence of transcriptional activation of target genes.[11, 12)] Although the underlying mechanism is not known, there is evidence that the SH3-binding domain and the C-terminal domain of p53 protein are necessary for inducing transactivation-independent apoptosis.[13–15)] It is likely that *p53* uses transactivation or direct protein-protein signaling or both to initiate apoptosis, depending on the cell types and experimental conditions.

ICE/CED-3 cysteine protease family (termed caspase) has been implicated in the execution of apoptosis induced by various stimuli. To date, more than ten members of the caspase family, including ICE, TX/ICH-2/ICErel-II, ICErel-III/TY, CPP32/apopain/Yama, Mch2, Mch3/ICE-LAP3/CMH-1, Mch4, Mch5/MACH/FLICE, Mch6 (ICE-LAP6) and ICH-1/NEDD-2, have been identified. Caspases are expressed as proenzymes which are proteolytically cleaved to produce active enzymes upon apoptotic stimulation.[16, 17)] The activated caspases can further activate other procaspases. Therefore, the multiple caspases are activated sequentially and ultimately cleave cellular protein substrates, leading to apoptotic morphology.[18–20)]

The role of caspase activation in *p53*-induced apoptosis has been investigated in several systems.[21–23)] However, in these systems, the induction of apoptosis depends on *p53* transactivation activity. It is still unknown whether *p53*-induced transactivation-independent apoptosis also requires caspase activation. Previously, we established J138V5C cells by transfecting human *ts p53* (138Val) into a human Jurkat T-cell line which contains heterozygous mutant *p53* and expresses no p53 protein.[24)] This *p53* gene acts as a mutant at 37.5°C, but acts as a wild-type *p53* and induces apoptosis at 32.5°C. In the present study, we observed that cycloheximide, which effectively blocked *de novo* protein synthesis, failed to prevent cells from undergoing apoptosis, suggesting that a transactivation-independent *p53* pathway operates in this system. We further investigated the role of caspase activation in *p53*-induced, transactivation-independent apoptosis. The results showed that both CPP32 and ICH-1 were activated upon activation of *p53*. The activity of CPP32 or some other DEVD-sensitive caspase(s) was necessary for cleavage of the ICH-1 precursor. We also explored whether the Fas pathway mediated *p53*-induced activation of caspase in this system. Although the absence of involvement of Fas in the *p53*-mediated apoptosis pathway was reported in *lpr* mouse after γ-irradiation,[25)] there has been no clear conclusion as to the involvement of Fas in the human

[1] To whom reprint requests should be addressed.
E-mail: tsuchida.mco@dent.tmd.ac.jp

system, partly due to the sequence difference between human *p53* and mouse *p53*,[26] and also due to different genetic backgrounds even within the human cells used for analysis. The inability of Fas and Fas ligand-neutralizing antibodies to block *p53*-induced apoptosis suggests that the Fas pathway is not essential for *p53*-induced apoptosis in this system.

MATERIALS AND METHODS

Cell culture and treatment of caspase inhibitor J138V5C and Jneo were established previously from a human Jurkat T-cell line.[24] The cells were grown in RPMI1640 medium containing 10% fetal calf serum (FCS) at 37.5°C for growth, or at 32.5°C for induction of apoptosis. For inhibitor treatment, Ac-DEVD-CHO (Peptide Institute, Osaka) was added to the culture medium at a final concentration of 100 μM and incubated at 37.5°C for 1 h before temperature shift-down.

DNA fragmentation analysis DNA was extracted as previously described.[27] Briefly, cells (2×10^6) were collected and washed with cold phosphate-buffered saline (PBS). The cell pellets were suspended in 40 μl of 0.2 *M* Na_2HPO_4/0.1 *M* citric acid (192:8, v/v) buffer and incubated at room temperature for 60 min. The suspensions were centrifuged at 2,000*g* for 30 min, then the supernatants were transferred to new tubes and treated with 0.02% Nonidet P-40 and RNase A (1 μg/ml) at 37°C for 30 min. Proteinase K (1 μg/ml) was added and the mixture was incubated at 50°C for 30 min. Aliquots (10 μl) were loaded on 1.5% agarose gel and electrophoresis was carried at 5 V/cm for 1 h in 1× TBE [89 m*M* Tris-borate, 2 m*M* EDTA (pH 8.0)] buffer. The DNA was stained with ethidium bromide and visualized under a UV illuminator.

Western blot analysis Cells cultured at permissive or non-permissive temperature were collected by centrifugation at low speed, washed with PBS, and lysed in RIPA buffer [1× PBS containing 1% Nonidet P-40, 0.5% sodium deoxycholate and 1% sodium dodecyl sulfate (SDS)] in the presence of proteinase inhibitors [0.1 μg/ml phenylmethanesulfonyl fluoride (PMSF) and 0.1 μg/ml aprotinin) and phosphatase inhibitor (1 m*M* sodium orthovanadate). DNAs were sheared by passage through 21 gauge needles and centrifuged at 15,000*g* for 20 min. The supernatant equivalent to 50 μg total protein was fractionated on 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a PVDF filter (Nihon Millipore, Tokyo) by using a semi-dry electroblotting system. The membrane was blocked in 1× TTBS [20 m*M* Tris-HCl (pH 7.6), 137 m*M* NaCl and 0.1% Tween-20] containing 5% skim milk and bands were detected with the following antibodies: anti-PARP (C-2-10) (Enzyme System Products, Dublin, OH), anti-CPP32 (clone 19) (Transduction Lab., Lexington, KY), ERK-2 (K-23), ICH-1 (H-19) and p21 (C-19) (Santa Cruz Biotechnology Inc., Santa Cruz, CA). The horseradish peroxidase-conjugated second antibody with the ECL detecting system (Amersham, Tokyo) was used to visualize immunoreactive proteins.

Fas and Fas ligand-neutralizing antibodies analysis Cells (5×10^4) were cultured in normal medium or medium containing apoptotic neutralizing anti-Fas antibody (clone ZB4) or anti-Fas ligand antibody (clone 4H9) (MBL Inc., Nagoya) at a concentration of 500 ng/ml for 1 h. Then the cells were either transferred to 32.5°C to induce apoptosis or maintained at 37.5°C as a control. The DNA fragmentation and cell viability were analyzed at different time points.

RESULTS

***p53*-induced apoptosis in Jurkat cells does not require *de novo* protein synthesis** *p53* can exert either transcriptional activation-dependent or -independent activity to induce apoptosis, depending on the cell types or experimental conditions. To elucidate the pathway by which *p53* induces apoptosis in Jurkat cells, we analyzed whether the translation inhibitor cycloheximide could block *p53*-induced apoptosis. As cycloheximide itself induced apoptosis at high concentration in J138V5C cells, we treated

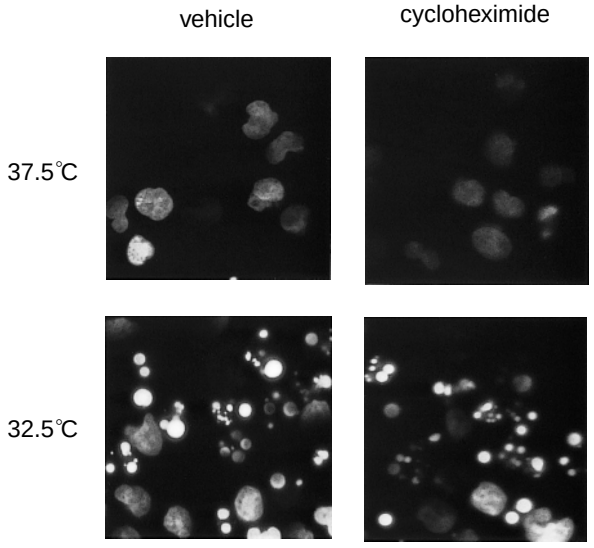

Fig. 1. *p53*-induced chromatin condensation in the absence of translation of *p53*-responsive genes. J138V5C cells (1×10^6) were treated with vehicle or cycloheximide (0.5 μg/ml, Sigma, St. Louis, MO) for 2 h before the culture was shifted to 32.5°C or maintained at 37.5°C. After 18 h of temperature shift-down, the cells were collected. They were fixed in 4% formaldehyde for 30 min, then stained with Hoechst 33342 (10 μM in PBS) and observed under a fluorescence microscope.

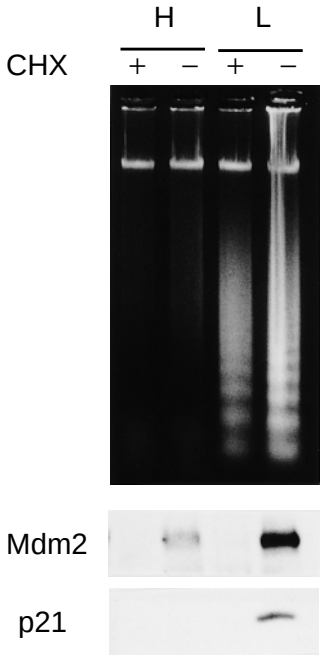

Fig. 2. *p53* induces apoptosis in the absence of translation of *p53*-responsive genes. J138V5C cells (1×10^6) were treated with cycloheximide or vehicle at 37.5°C for 2 h then split into two groups. One was left at 37.5°C (H) and the other was shifted to 32.5°C (L). After 18 h of temperature shift-down, the cells were collected and examined for DNA fragmentation (upper panel) and protein level of *p53*-responsive gene products, MDM2 and p21 (lower panel), as described in "Materials and Methods." CHX: cycloheximide.

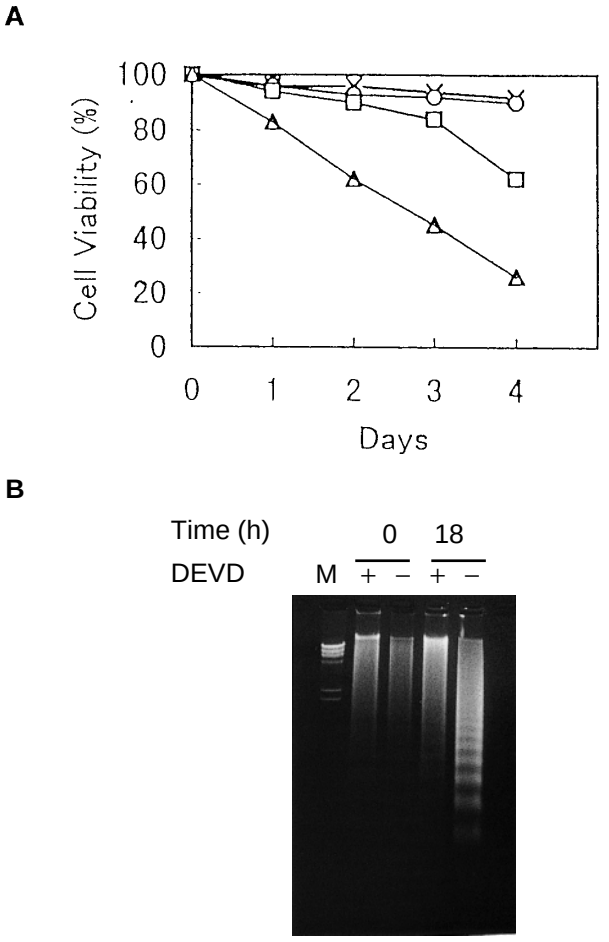

Fig. 3. Effect of caspase inhibitor on *p53*-induced apoptosis. A: Cells (5×10^4) were cultured in 24-well plates in the presence (+) or absence (–) of 100 μM DEVD at 37.5°C for 1 h, then either shifted to 32.5°C (L) or left at 37.5°C (H). The cell viability was determined by means of the trypan blue exclusion test at different time points. × H+, ○ H–, □ L+, △ L–. B: Cells (2×10^6) were treated with the caspase inhibitor DEVD as in A. DNA fragmentation was analyzed before (0) or 18 h (18) after temperature shift-down. *p53*-induced DNA fragmentation (18–) was blocked by caspase inhibitors (18+). No DNA fragmentation was detected in cells cultured at 37.5°C with (0+) or without (0–) inhibitors.

J138V5C cells with cycloheximide at a concentration of 0.5 μg/ml for 2 h before and during temperature shift-down for 18 h. The treatment failed to block *p53*-induced apoptosis at permissive temperature (32.5°C) as judged from the appearance of chromatin condensation and nuclear fragmentation (Fig. 1). At 18 h after temperature shift-down, 70% of cycloheximide-treated cells and 68.5% of untreated cells showed chromatin condensation and nuclear fragmentation. More than 90% of cells cultured at non-permissive temperature (37.5°C) were normal. DNA fragmentation in cycloheximide-treated cells was as extensive as in untreated cells (Fig. 2, upper panel). The result of western blotting showed that protein synthesis from two *p53*-responsive genes, *p21* and *mdm2*, was effectively blocked (Fig. 2, bottom panel). These results strongly suggested that the translation of *p53* responsive genes was not essential for *p53*-induced apoptosis.

Activation of caspase is essential for *p53*-induced apoptosis To investigate the involvement of caspases in *p53*-induced apoptosis, we first examined the effect of the CPP32-preferential tetrapeptide inhibitor Ac-DEVD-CHO. Upon temperature shift-down, J138V5C cells cultured without inhibitor showed extensive apoptosis as judged by trypan blue exclusion test and DNA fragmentation analysis. Treatment of cells with 100 μM Ac-DEVD-CHO effectively prevented the loss of viability and DNA fragmentation induced by *p53* (Fig. 3). These results indicated that CPP32 or some other DEVD-sensitive caspase(s) was essential for *p53*-induced apoptosis.

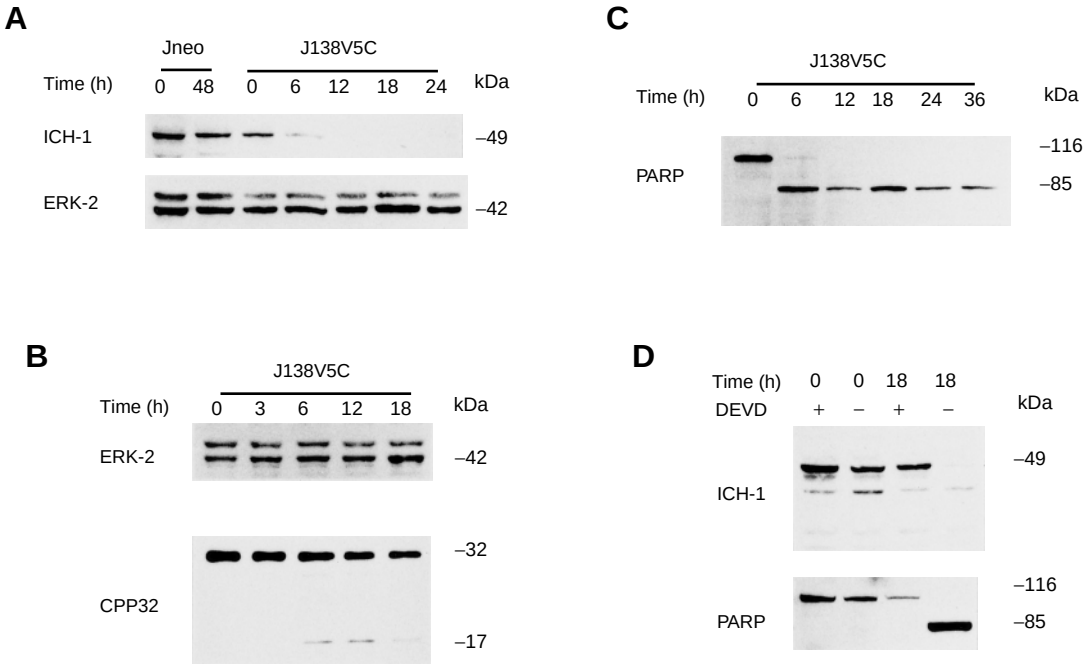

Fig. 4. Western blot analysis showing the activation of caspases. Cleavage of ICH-1 (A), CPP32 (B) and PARP (C). D: Ac-DEVD-CHO blocked the cleavage of ICH-1 and PARP. Western blotting was performed as described in "Materials and Methods." Time (h) represents the time period after temperature shift-down.

To elucidate further which of the caspases was involved in *p53*-induced apoptosis, we examined the cleavage of caspase precursors and caspase substrate by western blotting. ICH-1 is expressed as a 49 kDa proenzyme which is activated by multi-step cleavages.[22] Using an antibody against the prodomain, we detected the 49 kDa precursor in cells cultured at 37.5°C. This protein disappeared from cells cultured at 32.5°C for 6 h (Fig. 4A). CPP32 is synthesized as a 32 kDa precursor which is cleaved to 17 kDa and 12 kDa subunits upon activation.[17] Using a monoclonal antibody against the prodomain and 17 kDa subunit of CPP32, we detected a high level of 32 kDa CPP32 precursor in J138V5C cells at non-permissive temperature. This 32 kDa band decreased and the 17 kDa band appeared as early as 6 h after temperature shift-down (Fig. 4B). The 116 kDa nuclear enzyme PARP, a common substrate for multiple caspases, was almost completely cleaved to the 85 kDa subunit at the same time point (Fig. 4C). These observations suggested that both CPP32 and ICH-1 were activated in Jurkat cells undergoing *p53*-induced apoptosis.

To investigate the occurrence of the caspase activation cascade during *p53*-induced apoptosis, we examined the effect of the CPP32-preferential inhibitor Ac-DEVD-CHO on the activation of ICH-1. We found that Ac-DEVD-CHO completely blocked the cleavage of ICH-1 and PARP precursors (Fig. 4D), suggesting that CPP32 or some other DEVD-sensitive caspase was required for the activation of ICH-1.

Fas pathway is not essential for *p53*-induced apoptosis Activation of caspases via the Fas pathway has been investigated extensively.[28] The Fas pathway is essential for induction of apoptosis by anticancer drugs and *myc*, which may also require *p53* activation.[29–31] Although the transactivation of *p53*-responsive genes was not essential for *p53*-induced apoptosis, it is possible that *p53* might activate the Fas pathway in a transactivation-independent way. Based on this consideration, we examined whether Fas receptor and Fas ligand-neutralizing antibodies could block *p53*-induced apoptosis in this system. An agonistic anti-Fas antibody (CH11) induced apoptosis in J138V5C cells at non-permissive temperature, indicating that the Fas pathway is intact in this cell line. An antagonistic Fas antibody (ZB4) blocked CH11-induced apoptosis. However, at the same concentration, it failed to block apoptosis induced by *ts p53* in this system (Fig. 5). Morphological change, membrane blebbing and DNA fragmentation in ZB4-treated cells were all as extensive as in untreated cells. Furthermore, the anti-Fas ligand antibody (4H9), which had been shown to block Fas

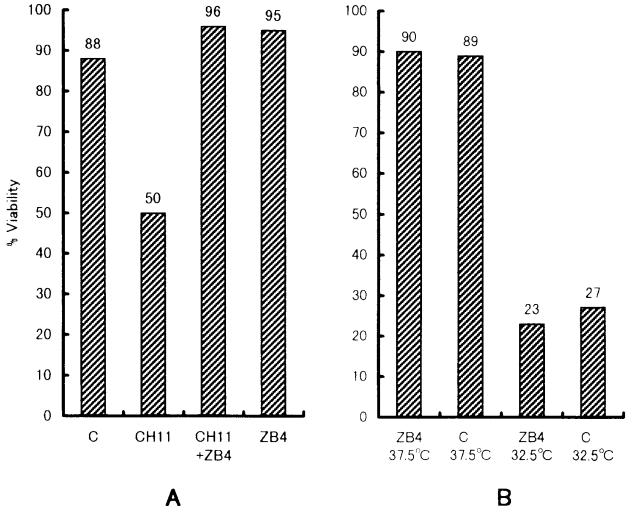

Fig. 5. Effect of *Fas*-neutralizing antibody on *p53*-induced apoptosis. A: Fas-neutralizing antibody (ZB4) blocked apoptosis induced by anti-Fas antibody (CH11) in J138V5C cells. Cells (5×10^4) were preincubated with ZB4 (500 ng/ml) before treatment with CH11 (400 ng/ml). The cell viability was analyzed by means of the trypan blue exclusion test at 24 h after the treatment with CH11. B: ZB4 failed to block *p53*-induced apoptosis. Cells were pretreated with ZB4 as in A, then either transferred to 32.5°C or maintained at 37.5°C for 4 days. The cell viability was analyzed by means of the trypan blue exclusion test.

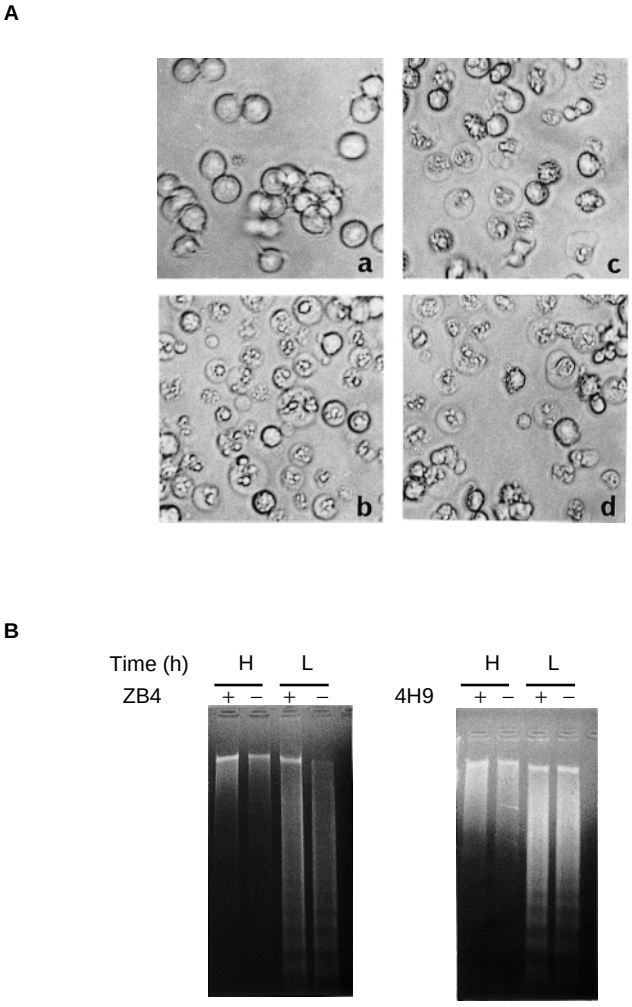

Fig. 6. Fas and Fas ligand-neutralizing antibodies failed to block *p53*-induced morphological change and DNA fragmentation in Jurkat cells. A: Cells (5×10^4) were cultured in normal medium (a, b), or medium containing 500 ng/ml ZB4 (c) or 400 ng/ml 4H9 (d) for 1 h. They were transferred to 32.5°C (b, c, d) or maintained at 37.5°C (a) for 24 h before the phase-contrast photomicrographs were taken (magnification 10×40). B: Cells treated (+) or untreated (–) with ZB4 or 4H9 were transferred to 32.5°C (L) or maintained at 37.5°C (H) for 24 h before DNA fragmentation analysis.

ligand-induced apoptosis, also failed to block *p53*-induced apoptosis (Fig. 6). These results suggested that the Fas pathway was not essential for *p53*-induced apoptosis in this system.

DISCUSSION

Overexpression and activation of human *ts p53* without other stimuli induce apoptosis in Jurkat cells. The previous experiment showed that several *p53*-responsive genes were up-regulated upon temperature shift-down.[24] However, the level of bax protein, one of the *p53*-responsive gene products, did not change significantly before and after temperature shift-down.[32] It has been reported that the temperature-sensitive *p53* 143Ala mutant can transactivate several *p53* target genes, but not apoptosis-associated *bax* and insulin-like growth factor-binding protein 3 gene promoter.[8] In addition, as shown in this study, the apoptosis was not prevented by a concentration of cycloheximide which effectively blocked the *de novo* synthesis of proteins, suggesting that transactivation activity was not essential for apoptosis. Although it is not known whether the translation of all known and unknown *p53* target genes was blocked to the same extent under our experimental conditions, the result that the number of apoptotic cells showed no significant difference in the presence of cycloheximide, as measured by Hoechst 33342 staining, further supported the possibility that transactivation-independent apoptosis was the major event in the present system. This result is consistent with and extends the earlier findings that UV induces *p53*-dependent apoptosis in the absence of protein synthesis,[11] and that transcriptionally inactive *p53* mutants retained the

ability to induce cell death.[12] The different apoptotic pathways induced by *p53* in different systems may result from the difference in cell types. The *p53* species employed may also be a contributing factor. In spite of the high homology, there are important structural and functional differences between different species of *p53*.[33, 34] For example, the murine p53 shares only 40% homology with human p53 in its SH3-binding domain (amino acid residues 62–91), which is important for *p53*-induced apoptosis.[13]

Caspase family members are expressed as proenzymes, which are cleaved and activated during the apoptotic process.[16, 17] Apoptosis induced by a given stimulus in a particular cell type may involve a selective activation of several caspases. It has been shown that *p53*-induced transactivation-dependent apoptosis involves activation of caspases. CPP32 seems to be a common mediator of apoptosis.[21–23, 35] In this study, we observed that the CPP32-preferential inhibitor Ac-DEVD-CHO effectively prevented *p53*-induced apoptosis, suggesting that CPP32 or some other DEVD-sensitive caspase(s) was required for *p53*-induced apoptosis in this system. The cleavage of CPP32 precursor to the 17 kDa active subunit further confirmed the essential role of CPP32 for *p53*-induced transactivation-independent apoptosis. Jurkat cells express a high level of ICH-1, and activation of ICH-1 has been shown to be involved in Fas and tumor necrosis factor (TNF) α induced-apoptosis in Jurkat cells.[36] The ICH-1 precursor was also cleaved during *p53*-induced apoptosis in our case, suggesting that the activation of ICH-1 is a common event in Jurkat cells undergoing apoptosis induced by various stimuli. The activation of CPP32 and ICH-1 preceded the morphological change and DNA fragmentation, which were relatively early events in *p53*-induced apoptosis in this system. These results demonstrated that caspases were also essential for *p53*-induced, transactivation-independent apoptosis. The sequential activation of CPP32-like caspases and ICH-1 had been suggested in Jurkat cells which underwent Fas and TNF-induced apoptosis.[36] As the CPP32-preferential peptide inhibitor Ac-DEVD-CHO, but not Ac-YVAD-CHO (data not shown), blocked the cleavage of ICH-1 precursor, we suggest the sequential activation of CPP32 or other DEVD-sensitive caspase(s) and ICH-1 in *p53*-induced apoptosis. This would be in agreement with the report that ICH-1 was a substrate for CPP32 *in vitro*.[18] To our knowledge, this is the first report showing that caspase is essential for *p53*-induced transactivation-independent apoptosis.

p53 may mediate the activation of caspase through transcriptional activation of *bax* in some systems.[22] However, in our system, apoptosis was induced by *p53* in the absence of protein synthesis from *p53* transcriptional target genes, suggesting that *p53*-induced apoptosis in this system is not initiated by up-regulated bax protein. This result also suggests that induction of other *p53*-responsive genes such as *Fas*, *IGF-BP3* and *p85* is not required for *p53*-induced apoptosis.[10, 37] The Fas/Fas ligand pathway is another route leading to activation of caspase.[28] To clarify whether *p53* may activate Fas/FasL through a pathway other than transcriptional activation, we blocked the Fas pathway with Fas and Fas ligand-neutralizing antibodies: both antibodies failed to block *p53*-induced morphological change, DNA fragmentation and loss of cell viability. The Fas death pathway was triggered by the binding of Fas ligand to Fas receptor. Both the Fas-neutralizing antibody (ZB4) and Fas ligand-neutralizing antibody (4H9) effectively blocked the activation of the Fas pathway in this and other systems.[38] These data indicated that *p53*-induced apoptosis does not involve the activation of the Fas pathway. This result is consistent with and extends the previous report that cells from Fas-deficient *lpr* mice were still sensitive to γ-irradiation-induced, *p53*-dependent apoptosis,[25] although γ-irradiation may have additional effects on apoptosis other than via the *p53*-dependent pathway. Taken together, our results indicate that *p53*-induced transactivation-independent apoptosis involves sequential activation of CPP32 or some other DEVD-sensitive caspase(s) and ICH-1, which is not mediated by the Fas pathway.

ACKNOWLEDGMENTS

We are very grateful to Dr. A. K. Munirajan for a critical reading of the manuscript. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

(Received August 13, 1998/Revised November 6, 1998/Accepted November 11, 1998)

REFERENCES

1) Green, M. S., Bennett, W. P., Hollstein, M. and Harris, C. C. Mutation in the p53 tumor suppressor gene: clue to cancer etiology and molecular pathogenesis. *Cancer Res.*, **54**, 4855–4878 (1994).
2) Levine, A. J. p53, the cellular gatekeeper for growth and division. *Cell*, **88**, 323–331 (1997).
3) Ko, L. J. and Prives, C. p53: puzzle and paradigm. *Genes Dev.*, **10**, 1054–1072 (1996).
4) El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, R., Parsons, R., Trent, J. M., Lin, D., Mercer, E., Kinzler, K. W. and Vogelstein, B. WAF1, a potential mediator of p53 tumor suppression. *Cell*, **75**, 817–825 (1993).

5) Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K. and Elledge, S. J. The p21 cdk-interacting protein cip1 is a potent inhibitor of G1 cyclin-dependent kinase. *Cell*, **75**, 805–816 (1992).
6) Xing, Y., Hanmon, G. J., Zhang, H., Casso, D., Kobayashi, R. and Beach, D. p21 is a universal inhibitor of cyclin kinase. *Nature*, **366**, 701–704 (1993).
7) Sabbatini, P., Lin, J., Levine, A. J. and White, E. Essential role for p53-mediated transcription in E1A-induced apoptosis. *Genes Dev.*, **9**, 2184–2192 (1995).
8) Friedlander, P., Haupt, Y., Prives, C. and Oren, M. A mutant p53 that discriminates between p53-responsive genes cannot induce apoptosis. *Mol. Cell. Biol.*, **16**, 4961–4971 (1996).
9) Miyashita, T. and Reed, J. C. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell*, **80**, 293–299 (1995).
10) Buckbinder, L., Talbott, R., Velasco-Miguel, S., Takenaka, I., Faha, B., Seizinger, B. R. and Kley, N. Induction of the growth inhibitor IGF-binding protein 3 by p53. *Nature*, **377**, 646–649 (1996).
11) Caelles, C., Helmberg, A. and Karin, M. p53 dependent apoptosis in the absence of transcriptional activation of p53-target genes. *Nature*, **370**, 220–223 (1994).
12) Haup, Y., Rowan, S., Shaulian, E., Vousden, K. H. and Oren, M. Induction of apoptosis in HeLa cells by trans-activation-deficient p53. *Genes Dev.*, **9**, 2170–2183 (1995).
13) Walker, K. K. and Levine, A. J. Identification of a p53 functional domain that is necessary for efficient growth suppression. *Proc. Natl. Acad. Sci. USA*, **93**, 15335–15340 (1996).
14) Chen, X., Ko, L. J. and Prives, C. p53 level, functional domains and DNA damage determine the extent of apoptotic response of tumor cells. *Genes Dev.*, **10**, 2438–2451 (1996).
15) Wang, X. W., Vermeulen, W., Coursen, J. D., Gibson, M., Lupold, S. E., Forrester, K., Xu, G., Elmore, L., Yeh, H., Hoeijmakers, J. J. and Harris, C. C. The XPB and XPD DNA helicases are components of the p53-mediated apoptosis pathway. *Genes Dev.*, **10**, 1219–1232 (1996).
16) Alnemri, E., Livingston, D. J., Nicholson, D. W., Salvesen, G., Thornberry, N. A., Wong, W. W. and Yuan, J. Y. Human ICE/CED-3 protease nomenclature. *Cell*, **87**, 171 (1996).
17) Tewari, M., Quan, L. T., O'Rourke, K., Desnoyer, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S. and Dixit, V. M. Yama/CPP32b, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell*, **81**, 801–809 (1995).
18) Srivnivasula, S. M., Ahmad, M., Fernandes-Alnemri, T., Litwack, G. and Alnemri, E. S. Molecular ordering of the Fas-apoptotic pathway: the Fas/apo-1 protease Mch5 is a CrmA-inhibitable protease that activates multiple Ced-3/ICE-like cysteine proteases. *Proc. Natl. Acad. Sci. USA*, **93**, 14486–14491 (1996).
19) Martin, S. J. and Green, D. R. Protease activation during apoptosis: death by a thousand cuts? *Cell*, **82**, 349–352 (1995).
20) Enari, M., Talanian, R. V., Wong, W. W. and Nagata, S. Sequential activation of ICE-like and CPP32-like protease during Fas-mediated apoptosis. *Nature*, **380**, 723–726 (1996).
21) Geley, S., Hartmann, B. L., Hattmannstorfer, R., Loffler, M., Ausserlechner, M. J., Bernhard, D., Sgonc, R., Strasser-Wozak, E. M. C., Ebner, M., Auer, B. and Kofler, R. p53-induced apoptosis in the human T-ALL cell line CCRF-CEM. *Oncogene*, **15**, 2429–2437 (1997).
22) Sabbatini, D., Han, J., Chiou, S. K., Nicholson, D. W. and White, E. Interleukin 1β converting enzyme-like proteases are essential for p53-mediated transcriptionally dependent apoptosis. *Cell Growth Differ.*, **8**, 643–653 (1997).
23) Lotem, J. and Sachs, L. Cytokine suppression of protease activation in wild-type p53-dependent and p53-independent apoptosis. *Proc. Natl. Acad. Sci. USA*, **94**, 9349–9353 (1997).
24) Yamato, K., Yamamoto, M., Hirano, Y. and Tsuchida, N. A human temperature-sensitive p53 mutant p53 val-138: modulation of the cell cycle, viability and expression of p53-responsive genes. *Oncogene*, **11**, 1–6 (1995).
25) Fuchs, E. J., Mckenna, K. A. and Bedi, A. p53-dependent DNA damage-induced apoptosis requires Fas/apo-1-independent activation of CPP32b. *Cancer Res.*, **57**, 2550–2554 (1997).
26) Soussi, T., Fromentel, C. C. and May, P. Structural aspects of p53 protein in relation to gene evolution. *Oncogene*, **5**, 945–952 (1990).
27) Gong, J., Traganos, F. and Darzynkiewilz, Z. A selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. *Anal. Biochem.*, **218**, 314–319 (1994).
28) Nagata, S. Apoptosis by death factor. *Cell*, **88**, 355–365 (1997).
29) Friesen, C., Herr, I., Krammer, P. H. and Debatin, K. M. Involvement of the CD95(Apo-1/Fas)receptor/ligand system in drug-induced apoptosis in leukemia cells. *Nat. Med.*, **2**, 574–577 (1996).
30) Fulda, S., Sieverts, H., Friesen, G., Her, I. and Debatin, K. M. The CD95(Apo-1/Fas) system mediates drug-induced apoptosis in neuroblastoma cells. *Cancer Res.*, **57**, 3823–3829 (1997).
31) Hueber, A., Zornig, M., Lyon, D., Suda, T., Nagata, S. and Evan, G. Requirement for the CD95 receptor-ligand pathway in c-*myc*-induced apoptosis. *Science*, **278**, 1305–1308 (1997).
32) Wang, J. F. Analysis of downstream effectors of p53 on cell growth arrest and apoptosis induced by a temperature-sensitive Val138 mutant. *J. Med. Dent. Sci.*, **45**, 141–149 (1998).
33) Hall, A. R. and Milner, J. Structural and kinetic analysis of p53-induced DNA complexes and comparison of human

and murine p53. *Oncogene*, **10**, 561–567 (1995).
34) Lane, D. P., Stephen, C. W., Midgley, C. A., Sparks, A., Hupp, T. R., Daniels, D. A., Greaves, R., Reid, A., Vojtesek, B. and Picksley, S. M. Epitope analysis of the murine p53 tumor suppressor protein. *Oncogene*, **12**, 2461–2466 (1996).
35) Salvesen, G. and Dixit, V. M. Caspase: intracellular signaling by proteolysis. *Cell*, **9**, 443–446 (1997).
36) Li, H., Bergeron, L., Cryns, N., Pasternack, M. S., Zhu, H., Shi, L., Greenberg, L. and Yuan, J. Activation of caspase-2 in apoptosis. *J. Biol. Chem.*, **272**, 21010–21017 (1997).
37) Yin, Y., Terauchi, Y., Solomon, G. G., Aizawa, S., Rangarajan, P. N., Yazaki, Y., Kadowaki, T. and Barrett, J. C. Involvement of p85 in p53-dependent apoptotic response to oxidative stress. *Nature*, **391**, 707–710 (1998).
38) Villunger, A., Egle, A., Kos, M., Hartmann, B. L., Geley, S., Kofler, R. and Greil, R. Drug-induced apoptosis is associated with enhanced Fas (Apo-1/CD95) ligand expression but independently of Fas (Apo-1/CD95) signaling in human T-acute lymphatic leukemia cells. *Cancer Res.*, **57**, 3331–3334 (1997).