Connective tissue growth factor (CTGF) is a member of an emerging CCN gene family that is implicated in various diseases associated with fibro-proliferative disorder including scleroderma and atherosclerosis. The function of CTGF in human cancer is largely unknown. We now show that CTGF induces apoptosis in the human breast cancer cell line MCF-7. CTGF mRNA was completely absent in MCF-7 but strongly induced by treatment with transforming growth factor β (TGF-β). TGF-β by itself induced apoptosis in MCF-7, and this effect was reversed by co-treatment with CTGF antisense oigonucleotide. Overexpression of CTGF gene in transiently transfected MCF-7 cells significantly augmented apoptosis. Moreover, recombinant CTGF protein significantly enhanced apoptosis in MCF-7 cells as evaluated by DNA fragmentation, Tdt-mediated dUTP biotin nick end-labeling staining, flow cytometry analysis, and nuclear staining using Hoechst 33258. Finally, recombinant CTGF showed no effect on Bax protein expression but significantly reduced Bcl2 protein expression. Taken together, these results suggest that CTGF is a major inducer of apoptosis in the human breast cancer cell line MCF-7 and that TGF-β-induced apoptosis in MCF-7 cells is mediated, in part, by CTGF.

Connective tissue growth factor (CTGF) is a member of an emerging gene family known as the CCN family (1), which includes CYR61 (cysteine-rich 61 kDa, TGF-β family member 10), Nov (nephroblastoma overexpressed) and the newly discovered WISP1/elm1, WISP2/scrC1p1, and WISP3 (2–5). CTGF was originally identified in condition medium of human umbilical vein endothelial cells (6) and plays a role in various human diseases including systemic scleroderma (7), atherosclerosis (8), renal diseases (9), hepatic fibrosis in biliary atresia (10), and malignant melanoma (11). A common finding in most of these diseases was a high level expression of CTGF in fibro-proliferative areas of affected tissues. In most cases, a direct correlation between high level CTGF expression and high levels of transforming growth factor-β (TGF-β) expression could be established. In fact, CTGF gene expression has been shown to be regulated by TGF-β (2).

TGF-β is a pleiotropic growth modulator with a wide variety of activities on different cell types. TGF-β stimulates proliferation of mesenchymal cells but inhibits that of endothelial (12) and epithelial cells (13). TGF-β is also known to inhibit the proliferation of a variety of cancer cell lines including the human breast cancer cell line MCF-7 (14, 15). In fact, the anticancer effect of tamoxifen has been attributed to indirect activation of TGF-β pathway and induction of apoptosis by TGF-β (16). Because CTGF is thought to be a mediator of TGF-β action, it is conceivable that CTGF may also inhibit cancer cell proliferation by inducing apoptosis in these cells. In the present study, we utilized antisense technology, transient overexpression techniques, and recombinant CTGF protein to gain insight into the biological function of CTGF in the MCF-7 breast cancer cell line. We now provide direct evidence that CTGF is pro-apoptotic in this cell line, suggesting an important role of CTGF in breast cancer biology.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Life Technologies, Inc. (Tokyo, Japan). Superf exquisite was purchased from Promega (Tokyo, Japan). Recombinant human CTGF was generously provided by Japan Tobacco (Osaka, Japan). Polyclonal anti-bcl2 and Bax antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell Culture—MCF-7, LL A549, CK201, and Mel D2–2 cancer cell lines were generously provided by the Department of Research, University Hospital of Basel, Basel, Switzerland. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (Tissue Culture Biologicals, Tulare, CA) in a 5% CO2 atmosphere at 37 °C. At 80% confluence, medium was replaced with serum-free DMEM supplemented with Insulin-transferin-selenium (Sigma, Tokyo, Japan) and used for all experiments except Northern analysis.

Northern Blot Analysis—Total cellular RNA was isolated using Trizol reagent (Life Technologies, Inc.) according to the manufacturer’s instructions. Total RNA (20 μg) was subjected to electrophoresis on 1% formaldehyde agarose gels and transferred to a nylon membrane (Highbond-N, Amersham Pharmacia Biotech). Blots were hybridized in QuickHyb (Strategene, CA) at 68 °C for 2 h with 32P-labeled cDNA probes prepared by random prime labeling (17). A 0.6-kilobase pair cDNA fragment contained within the open reading frame of CTGF was used as probe. Membranes were exposed to Bio-Max x-ray film (Eastman Kodak Co.) at ~70 °C for 2 h.

Cell Viability—Cell viability was evaluated using an MTT assay kit (Roche Molecular Biochemicals) according to the manufacturer’s instructions. Briefly, after treatment, cells were incubated for 4 h in the presence of MTT reagent and lysed with lysis buffer. After an overnight incubation, absorbance was measured at A690 nm. Nuclear Morphology—48 h after incubation with recombinant 5 μg/ml CTGF, both floating and trypsinized adherent cells were collected, washed with phosphate-buffered saline, fixed with 10% paraformaldehyde for 30 min, and incubated in Hoechst 33258 (Sigma) at room temperature for 30 min (final concentration, 30 μg/ml). Nuclear morphology was examined using fluorescence microscopy with standard
expression filters. To calculate the percentage of apoptotic cells, all cells from four random microscopic fields at 400× magnification were counted.

**DNA Fragmentation**—DNA fragmentation was measured using the Cell Death Detection ELISA PLUS kit (Roche Molecular Biochemicals) according to the manufacturer’s instructions. TdT-mediated dUTP biotin nick-end-labeling (TUNEL) was performed with the In Situ Cell Death Detection Kit from Roche Molecular Biochemicals according to the manufacturer’s instructions. To calculate the percentage of TUNEL positive cells, we counted all cells from four random microscopic fields at 100× magnification.

For flow cytometric analysis, both floating and trypsinized adherent cells were collected, washed with phosphate-buffered saline, and fixed with 70% ethanol. After fixation, cells were washed with phosphate-buffered saline and stained with propidium iodide for 20 min under subdued light. Stained cells were analyzed using fluorescence-activated cell sorter caliber (Becton Dickinson, Tokyo, Japan), and DNA content was calculated with Modfit software.

**Preparation of Antisense, Sense, and Scramble Oligonucleotide of CTGF**—18-mer CTGF sense and antisense oligonucleotides containing the initial ATG translation start site was synthesized (Amersham Pharmacia Biotech). Sequences were as follows: antisense, 5′-TACTGGCG-GCGGTCTAGCTTGCGGAC-3′ (18), and sense, 5′-ATGACCGCGCCGAGTA-3′ (18). An oligonucleotide containing a scrambled nucleotide sequence (5′-GTGCTAOGCTTGCGGAC-3′) was synthesized and used as an additional control. The synthetic oligonucleotides were directly added to the serum-free medium without any transfection compounds.

**CTGF Gene Overexpression in MCF-7 Cells**—To overexpress the human CTGF gene in MCF-7 cells, we constructed a mammalian expression vector (pCMV-CTGF) containing the complete open reading frame of the CTGF gene, driven by the CMV promoter (see Fig. 3A). To increase transcriptional efficiency, a 5′-polyadenylation signal sequence was used as a polyadenylation site. Mock transfected cells with pCMV vector alone (empty vector) were used as a control. Transient transfection was performed using superfect (Qiagen, Tokyo, Japan) according to the manufacturer’s protocol in serum-free DMEM. Transfection efficiency was evaluated by fluorescence microscopy in cells co-transfected with plasmid containing the green fluorescent protein (pEGFP-C1), which was obtained from CLONTECH. The average transfection efficiency using 1 μg of pEGFP-C1/105 cells was calculated to be about 55%.

**Western Blot Analysis of Bcl2 and Bax**—MCF-7 cells treated with 2.5 and 5 μg/ml recombinant CTGF for 48 h in serum-free DMEM were treated with lysis buffer containing SDS and mercaptoethanol. Primary antibody was used at 1:250 dilution. Cell lysates (20 μg) were subjected to 12.5% polyacrylamide gel (Ready Gel, Bio-Rad), transferred to polyvinylidene difluoride membranes (Bio-Rad), and incubated with 1:250 primary antibody for 1 h at room temperature as described previously (17, 19). Equal amount of protein loading was confirmed by Coomassie Brilliant Blue staining before blotting. The membranes were visualized using enhanced chemiluminescence (ECL kit; Amersham Pharmacia Biotech) and analyzed with image analysis software NIH-Image 1.60 for the Macintosh Power PC workstation.

**Detection of Caspase 3-like Activity**—Caspase 3-like activity was determined using the Caspase-3 Assay Kit (Biomol, Plymouth Meeting, PA) according to the manufacturer’s instructions. Briefly cells were lysed and centrifuged at 15,000 rpm for 10 min. Then 10 μl of supernatant was incubated with equal amount of substrate (ac-DEVD-p-nitroanilide), and O.D. was measured at 405 nm.

**Statistics**—Values are the means ± S.E. from four to six experiments. Statistical analysis of the data was performed using analysis of variance followed by Fisher’s test. p < 0.05 was considered significant.

**RESULTS**

**CTGF mRNA Expression in MCF-7 Cells**—Previous studies in our laboratory have shown that CTGF mRNA is not detectable by Northern blot or reverse transcription-polymerase chain reaction analysis in MCF-7 breast cancer cell lines grown in the presence of 10% fetal bovine serum.2 To investigate whether cancer cell lines derived from other organs express CTGF, we analyzed CTGF mRNA expression in the lung cancer-derived LK A549 cell line, the colon cancer-derived CK201 cell line, and the melanoma-derived Mel D2–2 cell line. As expected, MCF-7 grown in 10% fetal bovine serum did not express detectable levels of CTGF mRNA (Fig. 1). In contrast, LK A549, CK201, and Mel D2–2 expressed clearly detectable levels of CTGF mRNA. Surprisingly, CTGF gene expression in MCF-7 cells grown in the presence of 10% fetal calf serum was strongly induced by TGF-β (5 ng/ml) treatment for 48 h (Fig. 1). After 48 h of stimulation with 5 ng/ml TGF-β, CTGF mRNA levels also increased 7-fold in LK A549, 5-fold in CK201, and 1.7 fold in Mel D2–2 cells.

**Effect of CTGF Antisense Oligonucleotide on TGF-β-induced Apoptosis**—TGF-β induces apoptosis and inhibits MCF-7 cell growth, but the exact molecular mechanism is unclear (16). To investigate whether CTGF mediates the action of TGF-β in...
MCF-7 cells, we treated MCF-7 cells with 5–20 μg/ml CTGF antisense oligonucleotide. As shown in Fig. 2A, TGF-β (5 ng/ml) significantly reduced cell viability to 65 ± 5%, which was accompanied by an increase in histone-associated DNA fragmentation (Fig. 2B). Treatment with the CTGF antisense oligonucleotide dose-dependently reversed the effect of TGF-β (Fig. 2). Control experiments using 20 μg/ml scrambled or sense oligonucleotide did not affect TGF-β-induced cell death and DNA fragmentation (Fig. 2), suggesting a specific effect of CTGF antisense oligonucleotide in TGF-β-treated cells. We tested higher concentration up to 100 μg/ml, but we could not get any additional specific effects over 20 μg/ml.

Effect of CTGF Overexpression on Cell Viability and DNA Fragmentation—To investigate the effect of CTGF overexpression in MCF-7 cells, we transfected MCF-7 cells transiently with the expression plasmid pCMV-CTGF (1 μg DNA/10⁵ cells) (Fig. 3A). CTGF mRNA expression was confirmed using Northern blot analysis (Fig. 3B). 48 h after transfection, cell viability was significantly reduced by 35% (Fig. 4A), and histone-associated DNA fragmentation increased 3-fold as compared with controls (Fig. 4B). Mock transfection with pCMV alone did not have any effect on cell viability or DNA fragmentation.

Recombinant CTGF Induces Apoptosis in MCF-7—To investigate the direct effect of CTGF protein on MCF-7 cells, we used purified recombinant CTGF protein (10). Previous experiments using normal rat kidney fibroblasts confirmed that the recombinant CTGF protein we used was biologically active and increased cell death and DNA fragmentation (Fig. 2), suggesting a specific effect of CTGF antisense oligonucleotide in TGF-β-treated cells. We tested higher concentration up to 100 μg/ml, but we could not get any additional specific effects over 20 μg/ml.

Effect of Recombinant CTGF on Bcl2 and Bax—To investi-
gate whether CTGF-induced apoptosis in MCF-7 cells correlates with expression of proteins known to be involved in the apoptotic process, we performed Western blot analysis of Bcl2 and Bax in MCF-7 cells treated with 2.5 and 5 μg/ml recombinant CTGF for 48 h. As shown in Fig. 8A, recombinant CTGF dose-dependently reduced Bcl2 protein concentration in MCF-7 cells, but recombinant CTGF had no effect on Bax protein concentration in MCF-7 cells (Fig. 8B). In addition, we measured caspase 3-like activity in MCF-7 cells grown in 10% fetal calf serum. However, consistent with recent reports (20, 21), we could not detect caspase 3 activity in these cells (data not shown).

DISCUSSION

In the present study, we demonstrate that CTGF induces apoptosis in the human breast cancer cell line MCF-7. Our data suggest a potential role for CTGF in human breast cancer cells based on the following observations: 1) MCF-7 cells lacked CTGF mRNA expression when maintained in culture in the presence of fetal bovine serum, 2) recombinant CTGF protein dose-dependently reduced Bcl2 protein concentration in MCF-7 cells, but recombinant CTGF had no effect on Bax protein concentration in MCF-7 cells (Fig. 8B). In addition, we measured caspase 3-like activity in MCF-7 cells grown in 10% fetal calf serum. However, consistent with recent reports (20, 21), we could not detect caspase 3 activity in these cells (data not shown).

All members of the CCN gene family contain 38 totally conserved cysteine residues that are clustered in two segments (22 at the N-terminal region and 16 at the C-terminal region), separated by a region that varies in length and amino acid composition. Detailed analysis by Bork (1) revealed the presence of four distinct protein modules in this CCN protein family: 1) an insulin-like growth factor binding domain with the conserved putative IGF binding motif Gly-Cys-Gly-Cys-Cys-Xaa-Xaa-Cys (Xaa is any amino acid) located within the N-terminal portion of all IGF binding proteins, 2) a Von Willebrand factor type C repeat, which is thought to participate in oligomerization and protein complex formation, 3) a thrombospondin type 1 repeat, which is thought to be involved in binding to both soluble and matrix macromolecules, and in particular to sulfated glycoconjugates, and 4) a C-terminal module, which is homologous to slit, a protein involved in development of middling glia and commissural axon pathways in Drosophila that may represent a dimerization domain or be involved in receptor binding.

Although sequence homology between members of the CCN gene family is quite striking, functions of these proteins in vitro range from growth stimulation (6) to growth inhibition (23). Insights into the physiological functions and mechanism of action of this gene family are just starting to emerge. In particular, several lines of evidence support a role for the CCN gene family in tumorigenesis including reports that CTGF is overexpressed in pancreatic cancer (25), melanomas (11), and condrosarcoma (26). Angiogenic properties of CTGF are believed to contribute to tumor growth and vascularization. On the other hand, with other CCN family members, i.e. WISP1/Elm 1, expression is inversely related to the incidence of me-
expression. As mentioned above, CTGF gene expression is regulated by TGF-β (37), and in vascular smooth muscle cells CTGF mRNA increased 20-fold over basal level after stimulation with TGF-β. Grotendorst et al. (37) have recently found a novel TGF-β-responsive element with the consensus sequence 5′-GTGTCAAGGGGTC-3′ located between positions −162 and −128 of the CTGF promoter sequence. Point mutations in this responsive element resulted in a complete loss of CTGF induction. Therefore, CTGF is thought to be one of the downstream effectors of TGF-β (2). In MCF-7 cells CTGF mRNA expression is normally undetectable by Northern analysis but is strongly induced by treatment with TGF-β, suggesting that in these cells, CTGF is a downstream effector of TGF-β-induced apoptosis.

To clarify the downstream mechanism of CTGF-induced apoptosis in MCF-7 cells, we examined the protein expression levels of Bcl2 and Bax. Bcl2 protein is considered a major factor in the inhibition of apoptosis in many human cancers (38–40), and Bcl2 also regulates apoptosis in MCF-7. For example, apoptosis induced by melatonin followed by all trans-retinoic acids (35), Ro 41–5253 (41), lonidamine (42), and basic fibroblast growth factor (43) in MCF-7 is associated with down-regulation of Bcl2. Moreover treatment with melatonin followed by all trans-retinoic acids (35) or Ro 41–5253 (41) also increases TGF-β expression in MCF-7. Bax is a dominant negative inhibitor of Bcl2, and overexpression of Bax sensitizes MCF-7 to radiation-induced apoptosis (44). We now show that recombinant CTGF dose-dependently reduced Bcl2 but not Bax protein expression, suggesting that Bcl2 may represent a downstream target of CTGF in MCF-7 cells.

Recently, Janicke et al. (20, 21) found that TNF-α induced apoptosis in MCF-7 cells without inducing DNA fragmentation because of the lack of caspase-3 expression in these cells. Caspase-3 activity is thought to be essential for DNA fragmentation. In our hands, we also found that MCF-7 cells did not exhibit any caspase-3 activity (data not shown). Nevertheless, CTGF is capable of inducing DNA fragmentation and apoptosis in MCF-7 cells, suggesting that CTGF may utilize an alternative caspase pathway to induce apoptosis, i.e. caspase 6 or caspase 14 (45–47).

CTGF showed no effect on cell viability at concentrations below 1 μg/ml in contrast to TGF-β, which has activity in the nanomolar range. We found that the minimal effective concentration of recombinant CTGF was 5 μg/ml in both MCF-7 cells (Figs. 5 and 6) and normal rat kidney cells (data not shown). We suspect that the recombinant CTGF protein used in our experiments has an overall low biological activity and that because of the cysteine-rich nature of CTGF (total cysteine content is 11%), the percentage of correctly folded and biologically active recombinant protein may be very low (2).

In summary, we show that CTGF is pro-apoptotic in the MCF-7 human breast cancer cell line, and this effect is partly due to down-regulation of bcl2 protein. Furthermore, TGF-β-induced apoptosis in MCF-7 cells appears to be mediated by CTGF, suggesting that CTGF may play an important role in human breast cancer cell growth and may represent a new target for a therapeutic intervention.

REFERENCES
1. Bork, P. (1998) FEBS Lett. 327, 125–130
2. Oemar, B. S., and Lüscher, T. F. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 1483–1489
3. Babich, A. M., Chen, C. C., and Lau, L. F. (1997) Mol. Cell. Biol. 19, 2958–2966
4. Pennica, D., Swanson, T. A., Welsh, J. W., Roy, M. A., Lawrence, D. A., Lee, J., Brush, J., Tanyelh, L. A., Deuel, B., Lew, M., Watanabe, C., Cohen, R. L., Melhem, M. F., Finley, G. G., Quirke, P., Goddard, A. D., Hillian, K. J., Gurney, A. L., Botstein, D., and Levine, A. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14717–14722
5. Hashimoto, Y., Shindo-Okada, N., Tani, M., Nagamachi, Y., Takeuchi, K., Shiroishi, T., Toma, H., and Yokota, J. (1998) J. Exp. Med. 187, 289–296
6. Braddock, D. M., Igarashi, A., Potter, R. L., and Grotendorst, G. R. (1991) J. Cell Biol. 114, 1285–1294
7. Igarashi, A., Nishio, K., Kikuchi, K., Kato, S., Itoh, H., Grotendorst, G. R., and Takehara, K. (1995) J. Invest. Dermatol. 105, 280–284
8. Oemar, B. S., Werner, A., Garrié, J. M., Do, D. D., Godoy, N., Nauk, M., Marz, W., Rupp, J., Peck, M., and Lüscher, T. F. (1997) Circulation 95, 831–839
9. Ito, Y., Aten, J., Bende, R. J., Oemar, B. S., Rabelink, T. J., Weening, J. J., and Goldschmeding, R. (1998) Kidney Int. 53, 853–861
10. Tamasani, T., Kobayashi, H., Yonezuka, K., Sakamoto, S., Suzuki, K., Nakashima, K., Takigawa, M., and Miyano, T. (1998) Biochem. Biophys. Res. Commun. 251, 748–752
11. Kato, M., Kikuchi, K., Nishio, K., Kakinuma, T., Hayashi, N., Nanko, H., and
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Tamaki, K. (1998) Br. J. Dermatol. 139, 192–197

Tsukada, T., Eguchi, K., Mipita, K., Kawabe, Y., Kawakami, A., Matsuoka, N., Takashima, H., Minokami, A., and Nagataki, S. (1995) Biochem. Biophys. Res. Commun. 210, 1076–1082

Antoshina, E., and Ostrowski, L. E. (1997) In Vitro Cell Dev. Biol. Anim. 33, 212–217

Khosla, S., Oursler, M. J., Schroeder, M. J., and Eberhardt, N. L. (1994) Endocrinology 135, 1887–1893

Tatsuta, M., Ishii, H., Baba, M., Hirasawa, R., Iseki, K., Yano, H., Sakai, N., Uchida, H., and Nakaiumi, A. (1998) Br. J. Cancer 79, 857–861

Chen, H., Tritton, T. R., Kenny, N., Absher, M., and Chiu, J. F. (1996) Mol. Cell. Biol. 16, 139–148

Antoshina, E., and Ostrowski, L. E. (1997) J. Cell. Mol. Biol. 23, 153–161

Nicolau, C., Courjal, F., and Theillet, C. (1996) Clin. Cancer Res. 2, 1601–1606

Theile, M., Seitz, S., Arnold, W., Jandrig, B., Frege, R., Schlag, P. M., Haensch, W., Guiski, H., Winzer, K. J., Barrett, J. C., and Scherneck, S. (1996) Oncogene 13, 677–685

Choi, K. S., Lim, I. K., Brady, J. N., and Kim, S. J. (1998) Hepatology 27, 415–421

Perry, R. R., Kang, Y., and Greaves, B. R. (1995) Br. J. Cancer 72, 1441–1446

Warri, A. M., Huovinen, R. L., Laine, A. M., Martikainen, P. M., and Harkonen, P. L. (1993) J. Natl. Cancer Inst. 85, 1412–1418

Kyprianou, N., English, H. F., Davidson, N. E., and Isaacs, J. T. (1991) Cancer Res. 51, 162–166

Eck, K. M., Yuan, L., Duffy, L., Ram, P. T., Ayetey, S., Chen, I., Cohn, C. S., Reed, J. C., and Hill, S. M. (1998) Br. J. Cancer 77, 2129–2137

Sathiyamoorthy, N., Gilsdorf, J. S., and Wang, T. T. (1998) Anticancer Res. 18, 2449–2453

Grotendorst, G. R., Okochi, H., and Hayashi, N. (1996) Cell Growth Diff. 7, 469–480

Naumovski, L., and Cleary, M. L. (1994) Blood 83, 2261–2267

O'h Mori, T., Poduck, E. R., Nishio, K., Takahashi, M., Miyahara, Y., Takeda, Y., Kubota, N., Funayama, Y., Ogasawara, H., Ohira, T., et al. (1993) Biochem. Biophys. Res. Commun. 192, 30–36

Pietenpol, J. A., Papadopoulos, N., Markowitz, S., Willeon, J. K., Kinzler, K. W., and Vogelstein, B. (1994) Cancer Res. 54, 3714–3717

Toma, S., Isnardi, L., Raffo, P., Riccardi, L., Dastoli, G., Apel, C., LeMotte, P., and Bollag, W. (1998) Int. J. Cancer 78, 86–94

Del Bufalo, D., Bircocci, A., Soddu, S., Laudonio, N., D'Angelo, C., Sacchi, A., and Zupi, G. (1996) J. Clin. Invest. 98, 1165–1173

Wang, Q., Malof, P., Wang, H., Fenig, E., Stein, D., Nichols, G., Denny, T. N., Yalamol, J., and Wieder, R. (1998) Exp. Cell Res. 238, 177–187

Sakakura, C., Sweeney, E. A., Shirahama, T., Igarashi, Y., Hakomori, S., Nakatani, H., Tsujimoto, H., Imanishi, T., Ohgaki, M., Ohyama, T., Yamakata, J., Hagiwara, A., Yamaguchi, T., Sawai, K., and Takahashi, T. (1996) Int. J. Cancer 67, 101–105

Samejima, K., Svingen, P. A., Basi, G. S., Kottke, T., Mesner, P. W., Jr., Stewart, L., Durrieu, F., Poizier, G. G., Alnemri, E. S., Champoux, J. J., Kaufmann, S. H., and Earnshaw, W. C. (1994) J. Biol. Chem. 274, 4335–4340

Kurokawa, H., Nishio, K., Fukumoto, H., Tomonari, A., Suzuki, T., and Saijo, N. (1999) Oncol. Rep. 6, 33–37

Hu, S., Snipas, S. J., Vincenz, C., Salvesen, G., and Dixit, V. M. (1998) J. Biol. Chem. 273, 29648–29653