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Oligonucleosomal DNA Fragmentation in MCF-7 Cells Undergoing Palmitate-Induced Apoptosis

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Abstract—Oligonucleosomal fragmentation of nuclear DNA is the late-stage apoptosis hallmark. In apoptotic mammalian cells the fragmentation is catalyzed by DFF40/CAD DNase primarily activated by caspase 3 through the site-specific proteolytic cleavage of DFF45/ICAD. A deletion in the *casp3* gene of human breast adenocarcinoma MCF-7 results in lack of procaspase 3 in these cells. The absence of caspase 3 in MCF-7 leads to disability to activate oligonucleosomal DNA fragmentation in TNF-α induced cell death. In this study, sodium palmitate was used as an apoptotic stimulus for MCF-7. It has been shown that palmitate but not TNF-α induces both apoptotic changes in nuclei and oligonucleosomal DNA fragmentation in *casp3*-mutated MCF-7. Activation and accumulation of 40-50 kD DFF40-like DNases in nuclei of palmitate-treated apoptotic MCF-7 were detected by SDS-DNA-PAGE assay. Microsomal fraction of apoptotic MCF-7 does not contain any detectable DNases, but activates 40-50 kD nucleases when incubated with human placental chromatin. Furthermore, microsomes of apoptotic MCF-7 induce oligonucleosomal fragmentation of chromatin in a cell-free system. Both the activation of DNases and chromatin fragmentation are suppressed in the presence of the caspase 3/7 inhibitor Ac-DEVD-CHO. Microsome-associated caspase 7 is suggested to play an essential role in the induction of oligonucleosomal DNA fragmentation in *casp3*-deficient MCF-7 cells.

Key words: MCF-7, apoptosis, DNA fragmentation, caspase 3, caspase 7, caspase activated DNase

Apoptosis (a programmed cell death) plays an important role in maintenance of cell homeostasis at different stages of development of higher organisms. The cells that perish via apoptosis are characterized by a series of specific morphological features, such as dissipation of mitochondrial transmembrane potential ($\Delta\Psi_m$), depolarization and wrinkling of plasmatic membrane, condensation of cytoplasm and nucleus, and aggregation and oligonucleosomal fragmentation of chromatin [1-3]. The chromatin degradation into oligonucleosomal fragments occurs at late stages of apoptosis and is one of the general criteria for apoptotic cell death. The main role in this process belongs to the specialized caspase-dependent DNase, CAD/DFF40 [4, 5]. The activation of this enzyme occurs due to the site-specific proteolytic cleavage of the inhibitor ICAD/DFF45 comprising the complex CAD/ICAD (DFF40/DFF45) [4, 6-8]. Caspases 3 and 7 are known to cause this cleavage *in vitro* [9].

In human breast carcinoma cells MCF-7 this key enzyme of the apoptotic cascade is not expressed because of a deleted 47 bp in exon 4 of the *procaspase 3* gene. So, the apoptosis induced by tumor necrosis factor alpha (TNF-α) in MCF-7 cells is not accompanied by oligonucleosomal DNA fragmentation [10, 11].

Recently, it was found that apoptosis in MCF-7 is accompanied by DNA fragmentation when induced by a derivative of pyrrole-1,5-benzoxazepin (PNOX-6) [12], staurosporine [13], or paclitaxel [14]. Moreover, we found from studies on the toxic effect of human milk on MCF-7 cells that free fatty acids are the main cytotoxic factor, and apoptosis of MCF-7 induced either by natural milk or aliphatic fatty acids is accompanied by the oligonucleosomal fragmentation of nuclear DNA (unpublished data).

In apoptosis induced by sodium palmitate, the dissipation of mitochondrial transmembrane potential occurs due to the direct effect of sodium palmitate on the ADP/ATP antiporter [15-17]. In this case, the earliest

Abbreviations: TNF-α) tumor necrosis factor alpha; Chx) cycloheximide; Cyt) cytochrome; Apaf-1) apoptotic protease activating factor; CAD) caspase-activated deoxyribonuclease; ICAD) inhibitor of caspase-activated deoxyribonuclease; DFF) DNA fragmenting factor; PI) propidium iodide; Ac-DEVD-CHO) acetyl-Asp-Glu-Val-Asp aldehyde; MW) molecular weight.

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stages of apoptosis are characterized by the opening of non-selective mitochondrial pores and release of cytochrome *c* (Cyt *c*), apoptosis inducing factor (AIF), procaspases 2 and 9, and other proapoptotic molecules into the cytoplasm. A formation of a triple complex involving Cyt *c*, Apaf-1, and procaspase 9 in cytoplasm results in the proteolytic activation of procaspase 9. Caspase 9, in turn, can activate caspases 3 and 7 [3, 18, 19].

There is a strong structural and functional homology between the procaspases 7 and 3 [1, 19]. Procaspase 7 can specifically cleave DFF45 comprising the complex DFF40/DFF45 *in vitro*. In the absence of caspase 3 in MCF-7 cells, caspase 7 is the most probable candidate for the protease, whose activation makes irreversible (point of no return) the apoptotic cascade. Nevertheless, in both the apoptosis induced by TNF-α in MCF-7 cells and the apoptotic processes induced by Cyt *c* in cytoplasmic extracts of MCF-7, the level of caspase 7 activity appears to be insufficient for the devastative proteolysis of DFF45 [9]. Hence, the oligonucleosomal fragmentation of nuclear DNA in the MCF-7 apoptosis induced by either xenobiotics or fatty acids is indicative of other distinct but not yet investigated elements in the mechanism of apoptotic nuclear collapse and nuclear DNA destruction.

In the present study we have performed zymography of DNases activated during the MCF-7 apoptosis induced by sodium palmitate. We found that in palmitate-induced apoptosis DNases with MW of 40-50 kD are activated and accumulate in the nucleus. The activation of these DNases is caspase-dependent and occurs due to the functioning of a factor present in the microsomal fraction of apoptotic cells.

MATERIALS AND METHODS

MCF-7 cells were grown in the IMDM medium (Sigma, USA) supplemented with 10 mM L-glutamate, 40 μg/ml gentamicin, and 10% fetal calf serum (Biolot, St. Petersburg, Russia) in an atmosphere with 5% CO_2. Cell death was induced by either human recombinant TNF-α (100 ng/ml; NIIKTI BAV, Berdsk, Russia) or sodium palmitate (300-500 μM; Sigma). The amounts of apoptotic (annexin V^+/PI^-) and necrotic (annexin V^+/PI^+) cells were estimated from the content of cells being stained with FTC-annexin V conjugate (Promega, USA) in the presence of propidium iodide (Promega). The cell counting was conducted using a luminescence microscope (Aristoplan, Germany) equipped with an MTI CCD camera. To study morphological changes in nuclei, either cells grown on cover glasses or cell suspensions were incubated with apoptotic agents, fixed in 3% formaldehyde (PBS solution) followed by staining with 1 mg/ml propidium iodide, and then assayed by fluorescence microscopy. Isolation and electrophoretic analysis of DNA in 1.5% agarose gel were performed according to standard protocols [20].

To confirm a deletion in the *procaspase 3* gene, we amplified either MCF-7 cell DNA or control DNA from human placenta with the primers:

P1 - 5′-AAGTGCTTTTATGAAAATTCT-3′ and
P2 - 5′-AAGATCATACATGGAAGCGA-3′

corresponding to the areas 393-412 and 290-309 of the *caspase 3* transcript α mRNA (Gene Bank NM_004346.2) and the areas 14161-14180 and 14058-14077 of the human *caspase 3* gene (Gene Bank NT_022792.12) [10]. Deoxyribooligonucleotides were synthesized at the Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences. The amplification products were separated by electrophoresis in 8% non-denaturing polyacrylamide gel, followed by staining with 0.01% ethidium bromide.

Nuclei from the human placenta and MCF-7 cells were isolated by a method described elsewhere [21]. Microsomal fractions of MCF-7 were prepared as described by Chandler et al. [22]. To analyze the activation of apoptotic DNases in a cell-free system, the nuclei from human placenta (10 μg DNA) were suspended in 20 μl of 20 mM Tris-HCl buffer, pH 7.5, containing 150 mM NaCl and 0.1% 2-mercaptoethanol. Nuclear (5 μg DNA), microsomal (3 μg protein), or cytosolic (3 μg protein) fractions of apoptotic MCF-7 cells and 2 mM $MgCl_2$ were added to the suspension that was then incubated at 37°C for the period indicated in figure legends [9]. In experiments on the effect of the caspase 3 and 7 inhibitor Ac-DEVD-CHO on the activation of apoptotic DNases, we added either the inhibitor solution in DMSO (to 30 mM; Promega) or the equal amount of pure DMSO (controls).

For zymographic analysis of DNases, the protein samples were subjected to SDS-PAGE following Laemmli in 12% gels containing 0.2 mg/ml of salmon sperm DNA (Sigma) [23]. To restore DNases, the gel was incubated for 5 h in 50 mM Tris-HCl, pH 7.2, containing 3 mM $MgCl_2$ and 0.01% ethidium bromide. The stained DNA in gels was documented using a transilluminator and Olympus 2020Z digital camera.

RESULTS

Analysis of a deletion in the *procaspase 3* gene exon 4 of MCF-7 cells. It has been previously reported that a deletion of 43 bp in the MCF-7 *procaspase 3* gene results in failure of mRNA splicing and lack of procaspase 3 expression in these cells [10]. This deletion was found in several MCF-7 laboratory clones tested.

To confirm the *caspase 3* mutation in the laboratory clone of MCF-7 used in our experiments, we analyzed

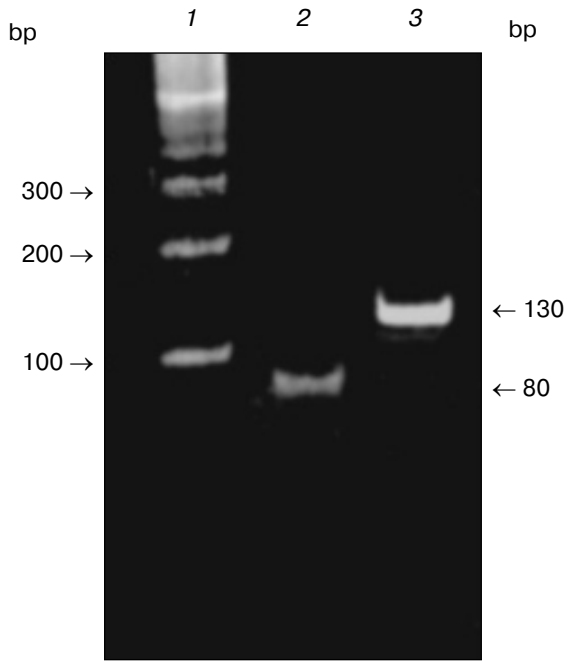

Fig. 1. Electrophoretic separation of the amplification products of MCF-7 DNA (*2*) and human placenta (*3*) in 8% nondenaturing polyacrylamide gel. Molecular weight markers (*1*). Amplification was conducted with the primers P1 and P2 flanking a deletion in the MCF-7 *caspase 3* gene. DNA was stained with ethidium bromide.

the DNA fragments amplified with the primers P1 and P2 flanking the deletion area in the *caspase 3* gene exon 4. We used both the human placenta DNA and DNA isolated from MCF-7 cells as templates. We found that in the case of MCF-7 only a fragment with the length of about 80 bp was amplified, which is about 50 bp shorter than the amplification product (with the same primers) of human placenta DNA (Fig. 1). Hence, this MCF-7 clone of adenocarcinoma is a *casp-3* mutant, alike the MCF-7 clones characterized earlier [10, 11].

DNA fragmentation in MCF-7 apoptosis. The incubation of MCF-7 in medium containing TNF-α/Chx leads to cytoplasm condensation, destruction of cell monolayer, and appearance of annexin V^+/PI^- cells detached from a plastic substrate (>60% after 8 h incubation). The TNF-α-induced apoptosis is accompanied by the condensation of nuclei without condensation of chromatin (Fig. 2a) or DNA fragmentation. These data are in good agreement with the previously reported data on the key role of caspase 3 in activation of DNase in the DFF40/DFF45 complex [9, 10].

The death of MCF-7 cells induced by sodium palmitate (70% are annexin V^+/PI^- cells and 10% are annexin V^+/PI^+ cells) is accompanied by the above-mentioned apoptotic features and also by the condensation of chromatin with formation aggregates or falx-structures on the periphery of the nucleus (Fig. 2a). Then cellular DNA undergoes hydrolysis to form nucleosomal fragments

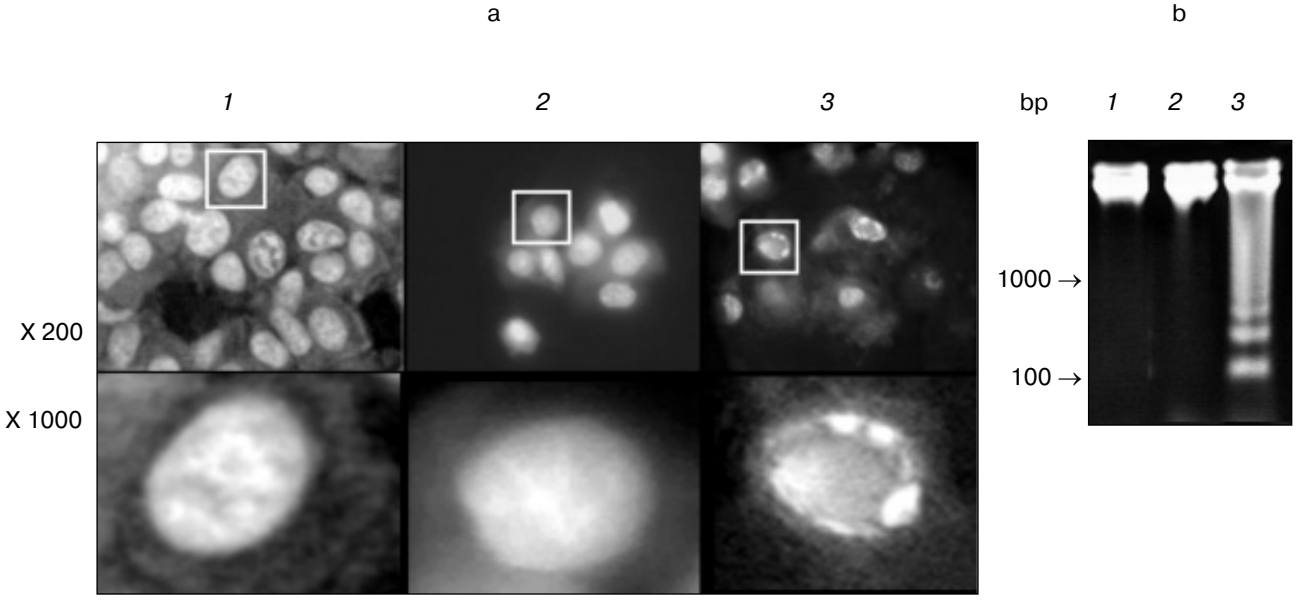

Fig. 2. a) Fluorescence microscopy of MCF-7 cells incubated for 12 h in the presence of TNF-α/Chx (*2*), palmitate (*3*), and with no inducer (*1*). Suspensions of cells detached from the plastic substrate (*2*, *3*) and cell monolayer (*1*) were used in the experiment. The cells were fixed in 3% formaldehyde and stained with PI. b) DNA fragmentation in MCF-7 cells in apoptosis induced by TNF-α/Chx (*2*) or sodium palmitate (*3*) or not subjected to any inducer (*1*). Electrophoresis of DNA was conducted in 1.8% agarose gel. DNA was stained with ethidium bromide.

(Fig. 2b, lane *3*). The hydrolysis of MCF-7 nuclear DNA results in di- and mononucleosomal fragments 30 h after the addition of sodium palmitate to the culture medium. Thus, the devastative activation of DNase(s) occurs, which leads to the both apoptotic changes in nuclei and oligonucleosomal DNA fragmentation when MCF-7 cells are incubated with toxic concentrations of long-chain fatty acids.

DNases in apoptotic MCF-7 cells. To perform a zymographic analysis of DNases activated by sodium palmitate, we separated proteins from different MCF-7 fractions by SDS-PAGE. The mobility of DNases in such a gel is revealed after the protein renaturation and DNA staining with ethidium bromide (Fig. 3a).

The data from DNA zymography suggest the accumulation of DNases with MW 40-50 kD in the MCF-7 nuclei (Fig. 3a, lane *3*), which is caused by sodium palmitate. Both DNases with MW 40-50 kD and an enzyme with MW 30 kD were detected in the cytoplasm fraction (Fig. 3a, lane *4*).

The chromatin DNA from human placenta undergoes nucleosomal fragmentation when incubated with nuclear fraction of MCF-7 cells whose apoptosis is induced by sodium palmitate (Fig. 3b, lane *3*). The cytoplasm fraction of those cells causes a nonspecific necrotic degradation of chromatin (Fig. 3b, lane *4*). The pattern of DNA fragments can be explained through the activity of cytosolic proteases that cleave nucleosomal proteins, thus making DNA available for DNases (Fig. 3a, lane *4*). It is worth mentioning that active DNases are not detectable in the nuclear fraction of MCF-7 cells dying under the influence of TNF-α (Fig. 3a, lane *2*), and this fraction does not cause the human placenta chromatin fragmentation (Fig. 3b, lane *2*). Hence, the activation and accumulation of DNases with MW of 40 and 50 kD occurs in palmitate-induced, but not in TNF-α/Chx-induced apoptosis. Both nuclear morphology in the late stage of apoptosis and the effect of nuclear MCF-7 fractions on the human placenta chromatin in a cell-free system indicate that DNases with MW 40-50 kD play a pivotal role in the process of oligonucleosomal DNA fragmentation.

Role of caspases in apoptotic fragmentation of MCF-7 DNA. Procaspase 7 is the closest structural and functional homolog of procaspase 3 [3, 18]. Unlike granzyme B and caspases 6 and 8, caspase 7 can specifically cleave the inhibitor DFF45 and activate the nuclease DFF40 *in vitro* [9]. However, only a negligible induction of caspase 7 occurs, which is insufficient for a considerable activation of DFF40 and triggering of DNA fragmentation, when apoptotic processes are induced in a cytoplasmic extract of MCF-7 in the presence of Cyt *c*/dATP [9]. The functionally active caspase 7 is known to be localized partly in mitochondrial and preferably in microsomal fraction of apoptotic cells [22].

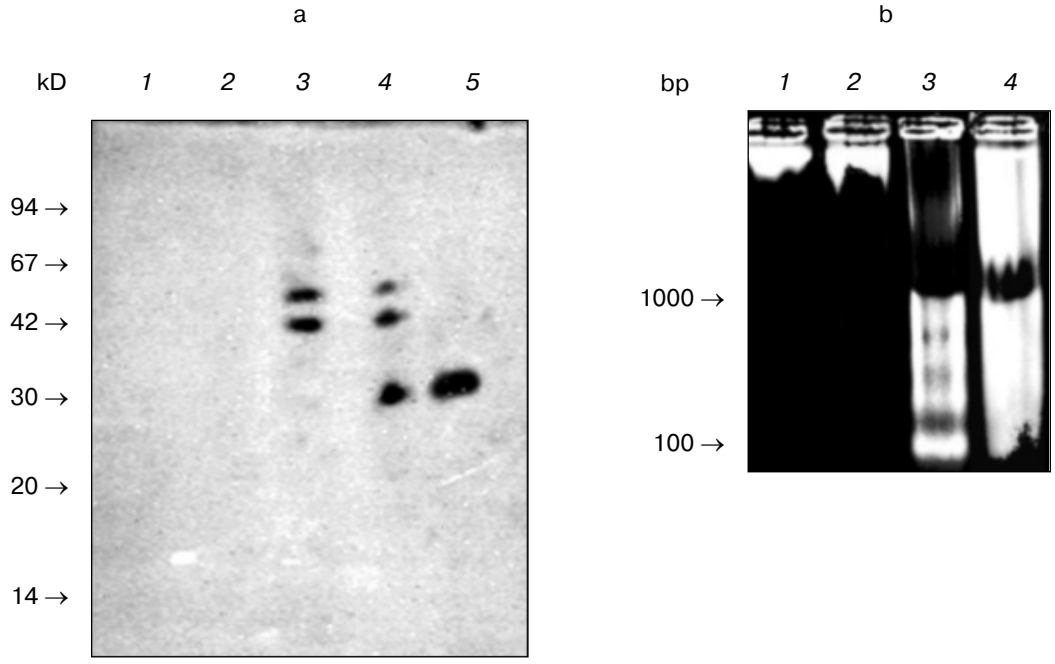

Fig. 3. a) Zymographic analysis of MCF-7 DNases in 12% DNA-SDS-polyacrylamide gel. Lanes: nuclear (*1-3*) and cytoplasmic (*4*) fractions of MCF-7 12 h after the challenge of apoptosis by TNF-α/Chx (*2*) or sodium palmitate (*3*, *4*), or without inducers (*1*); bovine DNase I was used as a control (*5*). b) Fragmentation of the human placenta DNA after 5 h of incubation in the presence of nuclear (*2*, *3*) or cytoplasmic (*4*) MCF-7 fraction, whose apoptosis was induced by TNF-α/Chx (*2*) or sodium palmitate (*3*, *4*); with no inducer (*1*).

To elucidate the role of caspase in the activation of DNases with MW 40-50 kD, we analyzed the effect of microsomal fraction of MCF-7 cells dying of sodium palmitate on the human placenta chromatin. The microsomal fraction of apoptotic MCF-7 cells did not contain the active DNases (Fig. 4a, lane *1*), but was able to challenge the oligonucleosomal fragmentation of chromatin in a cell-free system (Fig. 4b). As it takes place, DNases with MW 40-50 kD become active (Fig. 4a, lane *3*).

It is worth noting that the microsomal fraction of MCF-7 cells whose apoptosis is induced by TNF-α/Chx does not cause DNA fragmentation (Fig. 4b, lane *2*). Hence, the microsomal fraction of MCF-7 dying of fatty acids contains a DNase activator responsible for the oligonucleosomal DNA fragmentation.

Along with caspase 7, procaspase 12 was also found in the microsomal fraction [24]. Being activated in stress-induced apoptosis, the proteolytic fragments of caspase 12 could be found in cytoplasm [25] and/or nucleus [26] of dying cells. It is known that caspase 12 does not belong to the executing caspase family and differs significantly from caspases 3 and 7 by its substrate specificity [18].

To determine whether the DEVDase activity of caspase 7 comprising the microsomal fraction of apoptotic MCF-7 cells is essential for the activation of DNases with MW 40-50 kD, we analyzed the effect of the caspase 3/7 inhibitor Ac-DEVD-CHO on the activating properties of the microsomal fraction of the same cells. Using DNA-zymographic analysis in denaturing SDS-polyacrylamide gel, we found that in the presence of 50 μM Ac-DEVD-CHO tetrapeptide the microsomal fraction loses its ability to activate apoptotic DNases (Fig. 4a, lane *2*). Furthermore, Ac-DEVD-CHO represses the ability of microsomal MCF-7 fraction to cleave the human placenta DNA (Fig. 4b, lane *4*).

The tetrapeptide Ac-DEVD-CHO, due to its aldehyde residue, is a chemically active substance that can modify nucleophilic groups of both intracellular components and outer cell membrane. It hardly penetrates the cell membrane from the culture medium into the cytoplasm [27]. According to our observations, Ac-DEVD-CHO is a toxic agent: it causes a necrotic death of MCF-7 cells, which is accompanied by nonspecific degradation of nuclear DNA. Nonetheless, when MCF-7 cells died of palmitate in the presence of 200 μM Ac-DEVD-CHO, no activation of DNases with MW 40-50 kD was observed.

Our data presented here along with previously reported data on devastative site-specific cleavage of

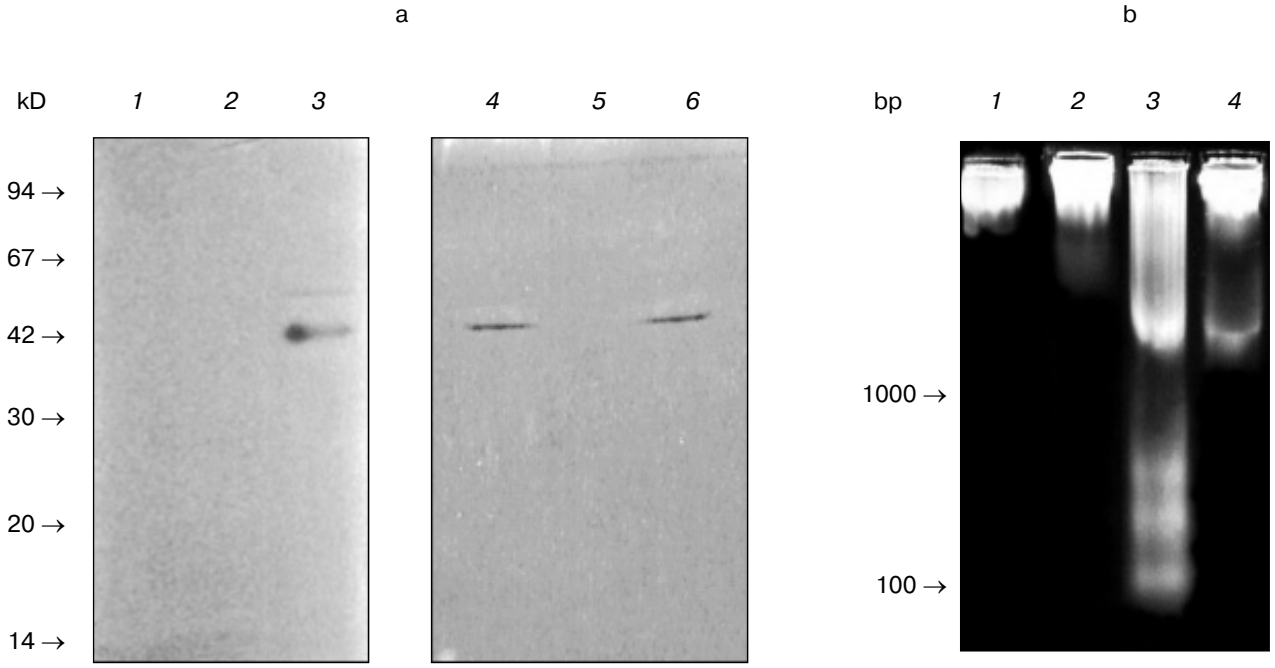

Fig. 4. a) Zymographic analysis of the activation of apoptotic DNases in a cell-free system using 12% DNA-SDS-polyacrylamide gel. Human placenta chromatin was incubated for 5 h at 37°C with no effector (*1*) or in the presence of intact (*3*, *4*), treated with Ac-DEVD-CHO/DMSO (*5*), or treated with DMSO only (*6*) microsomal fraction of apoptotic MCF-7. *2*) Intact microsomal fraction of apoptotic MCF-7 incubated under the same conditions. The apoptosis in MCF-7 was induced by sodium palmitate. After renaturation of DNases, DNA was stained with ethidium bromide. b) Electrophoretic analysis of human placenta DNA in 1.5% agarose gel after 5 h of incubation at 37°C with either no effectors (*1*) or in the presence of microsomal fractions of apoptotic MCF-7 (*2*-*4*). The death of MCF-7 was induced by either TNF-α/Chx (*2*), sodium palmitate (*3*), or sodium palmitate in the presence of Ac-DEVD-CHO (*4*). DNA in the gel was stained by ethidium bromide.

DFF45/ICAD in mitochondrion-induced MCF-7 apoptosis [14] suggest that it is caspase 7 that is responsible for the activation of apoptotic DNases and induction of the nuclear DNA fragmentation in *casp3*-mutant MCF-7.

DISCUSSION

As McGee and coauthors reported previously, the pyrrolo-1,5-benzoxazepin derivative PBOX-6 can challenge apoptotic death in MCF-7 cells accompanied by the oligonucleosomal DNA fragmentation [12]. The high level of DEVDase activity in lysate of cells incubated with PBOX-6 is significantly higher than that in cells undergoing TNF-α-induced apoptosis, thus suggesting that caspase 7 plays a key role in the induction of DNA fragmentation. The apoptosis of MCF-7 caused by the protein kinase inhibitor staurosporine is characterized by a low level of DEVDase activity in cytoplasm extract, but is accompanied by z-VAD-fmk-dependent fragmentation of nuclear DNA [13]. Both in the cases of PBOX-6- and staurosporine-induced apoptoses of MCF-7, only indirect evidences are available on mechanisms triggering apoptosis, and data which allow considering the nature of apoptotic nucleases responsible for the oligonucleosomal cleavage of nuclear DNA are absent.

In the present study we used sodium palmitate and TNF-α as inducers of apoptosis in the *casp3*-mutant MCF-7. The caspase cascade in TNF-α-mediated cell death is known to be initiated by caspase 8, but not by caspase 9 [11]. When apoptosis is induced by sodium palmitate, the initiation is realized via a release of mitochondrial factors into the cytoplasm, and the activation of caspase 9 is a limiting stage of the caspase cascade [15-19]. Distinct pathways of the cascade initiation lead to the significant differences in degrees of nuclear alterations and DNA fragmentation in the MCF-7 cells in the late stages of apoptosis (Fig. 2).

Earlier, using zymographic analysis of nucleases in DNA-polyacrylamide gel, Zhang et al. [23] demonstrated that apoptosis in Jurkat cells, which is caused by polyclonal antibodies against CD95 (Apo-1/Fas), is accompanied by accumulation of DNases with MW 42, 45, and 50 kD (NP42-50) in nuclei. Gu and Schlossman have shown a close similarity between the nucleases NP42-50 and caspase-induced DNase [28]. Moreover, the role of mitochondrial endonuclease G (30 kD) in the apoptotic fragmentation of nuclear DNA has been comprehensively studied. Although the specific activity of this endonuclease is significantly less than that of DFF40/CAD, its release from mitochondria and transport into the nucleus are caspase-independent processes [31]. Therefore, the mechanism involving endonuclease G might be considered as an alternative pathway of DNA fragmentation in the MCF-7 cells.

Cytoplasmic DNases activated in palmitate-induced apoptosis of MCF-7 cells correspond in their electrophoretic mobility in SDS-polyacrylamide gel to proteins with MW of ~30, 40, and 50 kD. The enzymes with MW 40 and 50 kD, but not the 30-kD nuclease, are present in nuclear fraction of apoptotic MCF-7 (Fig. 3). Both the activation of DNases and their accumulation in the nucleus during the palmitate-induced, but not TNF-α/Chx-induced apoptosis are accompanied by chromatin condensation and oligonucleosomal DNA fragmentation (Fig. 2). These data, along with known ability of executing caspase 3/7 inhibitor Ac-DEVD-CHO to repress the activation of DNases with MW of 40 and 50 kD (Fig. 4), allow attributing these nucleases to the caspase-activated DNase family DFF40/CAD.

Since the activation of DNases with MW of 40 and 50 kD is a caspase-dependent process, one may conclude that in palmitate-induced apoptosis another not yet described cascade of apoptotic events is realized, which is triggered by mitochondria and then, as the apoptosis develops, caspase 7 is activated and accumulates in microsomes, and at the last stage of apoptosis the nuclear DNA is condensed and undergoes oligonucleosomal fragmentation due to the action of DNases with MW of 42-50 kD.

Nagata et al. [32] found recently that the inactive CAD–ICAD complex is present not only in cytoplasmic (S-100), but also in microsomal (P-100) fractions of viable murine thymocytes. The cleavage of ICAD during the apoptotic death of thymocytes is accompanied by a "migration" of inactive CAD from the microsomal fraction to the nuclear and cytoplasmic fractions of dying cells. Taking into account that caspase 7 accumulates in microsomal fraction in the course of its activation [25], one can hypothesize that in apoptosis the endoplasmic reticulum plays a role of an executing portal facilitating a rapprochement of the active caspase 7 and its substrate, the CAD–ICAD complex. The effect of caspase 7 accumulation and co-localization with the substrate CAD–ICAD in endoplasmic reticulum *in vivo* might therewith compensate the low activity of caspase 7 observed in activation of CAD/DFF40 in the cell-free system *in vitro*.

This hypothesis in confirmed by the ability of microsomal fraction of apoptotic MCF-7 cells to activate nucleases with MW of 40-50 kD and to cause the fragmentation of chromatin (Fig. 4). Additional evidence is the data of Kottke [14] on the high cleavage degree of caspase 3 and 7 substrates on the background of low DEVDase activity in cytoplasmic extract of MCF-7 during paclitaxel-induced apoptosis. In the latter case, like under the action of fatty acids, the caspase cascade initiation takes place due to the release of mitochondrial Cyt *c* into the cytoplasm. The activation of caspases 9 and 7, condensation of chromatin, and oligonucleosomal chro-

matin fragmentation also become observed. Since this process is accompanied by the devastative cleavage of DFF45/ICAD, one can suppose that it is caspase 7 that is responsible for starting of apoptotic degradation of chromatin in the *casp-3*-deficient MCF-7 cells.

DNA fragmentation is the irreversible step of the apoptotic process in dying cells. The present data set is another argument that caspase 7, like caspase 3, can play a role of central element in the suicide cascade controlling functionality and dynamic stability of cell population in a living organism.

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REFERENCES

1. Hocker, G. (2000) *Cell Tissue Res.*, **301**, 5-17.
2. Cryns, V., and Yuan, J. (1998) *Genes Dev.*, **12**, 1551-1557.
3. Cohen, G. M. (1997) *Biochem. J.*, **326**, 1-16.
4. Nagata, S. (2000) *Exp. Cell Res.*, **256**, 12-18.
5. Counis, M. F., and Torriglia, A. (2000) *Biochem. Cell Biol.*, **78**, 405-414.
6. Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A., and Nagata, S. (1998) *Nature*, **391**, 43-50.
7. Liu, X., Zou, H., Slaughter, C., and Wang, X. (1997) *Cell*, **89**, 175-184.
8. Halenbeck, R., MacDonald, H., Roulston, A., Chen, T. T., Conroy, L., and Williams, L. T. (1998) *Curr. Biol.*, **8**, 537-540.
9. Wolf, B. B., Schuler, M., Echeverri, F., and Green, D. R. (1999) *J. Biol. Chem.*, **274**, 30651-30656.
10. Janicke, R. U., Sprengart, M. L., Wati, M. R., and Porter, A. G. (1998) *J. Biol. Chem.*, **273**, 9357-9360.
11. Janicke, R. U., Ng, P., Sprengart, M. L., and Porter, A. G. (1998) *J. Biol. Chem.*, **273**, 15540-15545.
12. McGee, M. M., Hyland, E., Campiani, G., Ramunno, A., Nacci, V., and Zisterer, D. M. (2002) *FEBS Lett.*, **515**, 66-70.
13. Mooney, L. M., Al-Sakkaf, K. A., Brown, B. L., and Dobson, P. R. (2002) *Br. J. Cancer*, **87**, 909-917.
14. Kottke, T. J., Blajeski, A. L., Meng, X. W., Svingen, P. A., Ruchaud, S., Mesner, P. W., Jr., Boerner, S. A., Samejima, K., Henriquez, N. V., Chilcote, T. J., Lord, J., Salmon, M., Earnshaw, W. C., and Kaufmann, S. H. (2002) *J. Biol. Chem.*, **277**, 804-815.
15. De Pablo, M. A., Susin, S. A., Jacotot, E., Larochette, N., Costantini, P., Ravagnan, L., Zamzami, N., and Kroemer, G. (1999) *Apoptosis*, **4**, 81-87.
16. Ostrander, D. B., Sparagna, G. C., Amoscato, A. A., McMillin, J. B., and Dowhan, W. (2001) *J. Biol. Chem.*, **276**, 38061-38067.
17. Boya, P., Roques, B., and Kroemer, G. (2001) *EMBO J.*, **20**, 4325-4331.
18. Slee, E. A., Harte, M. T., Kluck, R. M., Wolf, B. B., Casiano, C. A., Newmeyer, D. D., Wang, H. G., Reed, J. C., Nicholson, D. W., Alnemri, E. S., Green, D. R., and Martin, S. J. (1999) *J. Cell Biol.*, **144**, 281-292.
19. Chang, H. Y., and Yang, X. (2000) *Microbiol. Mol. Biol. Rev.*, **64**, 821-846.
20. Kaufmann, S. H., Mesner, P. W., Jr., Samejima, K., Tone, S., and Earnshaw, W. C. (2000) *Meth. Enzymol.*, **322**, 3-15.
21. Rickwood, D., Messent, A., Patel, D., Hinton, R. H., and Mullock, B. M. (1997) in *Subcellular Fractionation – A Practical Approach* (Graham, J. M., and Rickwood, D., eds.) Oxford University Press, Oxford, UK, pp. 75-76.
22. Chandler, J. M., Cohen, G. M., and MacFarlane, M. (1998) *J. Biol. Chem.*, **273**, 10815-10818.
23. Zhang, C., Robertson, M. J., and Schlossman, S. F. (1995) *Cell. Immunol.*, **165**, 161-167.
24. Nakagawa, T., Zhu, H., Morishima, N., Li, E., Xu, J., Yankner, B. A., and Yuan, J. (2000) *Nature*, **403**, 98-103.
25. Kilic, M., Schafer, R., Hoppe, J., and Kagerhuber, U. (2002) *Cell Death Differ.*, **9**, 125-137.
26. Fujita, E., Kouroku, Y., Jimbo, A., Isoai, A., Maruyama, K., and Momoi, T. (2002) *Cell Death Differ.*, **9**, 1108-1114.
27. Slee, E. A., Zhu, H., Chow, S. C., MacFarlane, M., Nicholson, D. W., and Cohen, G. M. (1996) *Biochem. J.*, **315**, 21-24.
28. Gu, J., Dong, R. P., Zhang, C., McLaughlin, D. F., Wu, M. X., and Schlossman, S. F. (1999) *J. Biol. Chem.*, **274**, 20759-20762.
28. Li, L. Y., Luo, X., and Wang, X. (2001) *Nature*, **412**, 95-99.
30. Van Loo, G., Schotte, P., van Gurp, M., Demol, H., Hoorelbeke, B., Gevaert, K., Rodriguez, I., Ruiz-Carrillo, A., Vandekerckhove, J., Declercq, W., Beyaert, R., and Vandenabeele, P. (2001) *Cell Death Differ.*, **8**, 1136-1142.
31. Van Loo, G., Saelens, X., van Gurp, M., MacFarlane, M., Martin, S. J., and Vandenabeele, P. (2002) *Cell Death Differ.*, **9**, 1031-1042.
32. Nagata, T., Kishi, T., Liu, Q. L., Matsuda, T., Imanaka, T., Tsukada, K., Kangl, D., and Muraguchi, A. (2002) *Immunology*, **105**, 399-406.