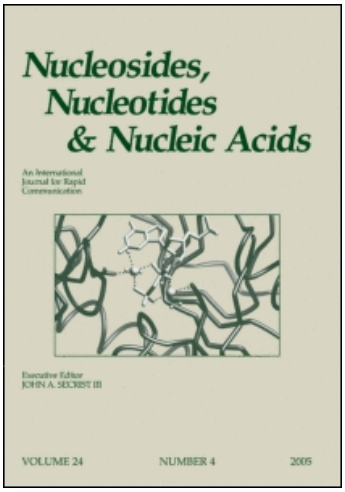

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Oligonucleosome DNA Fragmentation of Caspase 3 Deficient MCF-7 Cells in Palmitate-Induced Apoptosis

D. V. Semenov[a]; P. A. Aronov[a]; E. V. Kuligina[a]; M. O. Potapenko[a]; V. A. Richter[a]
[a] Novosibirsk Institute of Bioorganic Chemistry, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

Online publication date: 10 July 2004

To cite this Article Semenov, D. V. , Aronov, P. A. , Kuligina, E. V. , Potapenko, M. O. and Richter, V. A.(2004) 'Oligonucleosome DNA Fragmentation of Caspase 3 Deficient MCF-7 Cells in Palmitate-Induced Apoptosis', Nucleosides, Nucleotides and Nucleic Acids, 23: 6, 831 — 836
To link to this Article: DOI: 10.1081/NCN-200026024
URL: http://dx.doi.org/10.1081/NCN-200026024

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 6 & 7, pp. 831–836, 2004

Oligonucleosome DNA Fragmentation of Caspase 3 Deficient MCF-7 Cells in Palmitate-Induced Apoptosis

D. V. Semenov,* P. A. Aronov, E. V. Kuligina, M. O. Potapenko, and V. A. Richter

Novosibirsk Institute of Bioorganic Chemistry, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

ABSTRACT

Oligonucleosomal fragmentation of nuclear DNA is the late stage hallmark of the apoptotic process. In mammalian apoptotic cells fragmentation is catalyzed by DFF40/CAD DNase. DFF40/CAD primary activated through site-specific proteolytic cleavage by caspase 3. The absence of caspase 3 in MCF-7 leads to lack of oligonucleosomal DNA fragmentation under numerous apoptotic stimuli. In this study it was shown that palmitate induces apoptotic changes of nuclei and oligonucleosomal DNA fragmentation in casp3 deficient MCF-7. Activation and accumulation of 40–50 kDa DFF40 like DNases in nuclei and cytoplasm of palmitate-treated MCF-7 were detected by SDS-DNA-PAGE assay. Microsomes of apoptotic MCF-7 activate 40–50 kDa nucleases when incubated with human placental chromatin and induce oligonucleosomal fragmentation of chromatin in cell free system. Both DNases activation and chromatin fragmentation are suppressed in presence of caspase 3/7 inhibitor Ac-DEVD-CHO. Microsome associated caspase 7 is suggested to play the principal role in induction of oligonucleosomal DNA fragmentation of casp3 defitient MCF-7.

Key Words: MCF-7; Apoptosis; DNA fragmentation; Caspase 3; Caspase 7; Caspase activated DNAse.

*Correspondence: D. V. Semenov, Novosibirsk Institute of Bioorganic Chemistry, Siberian Branch of the Russian Academy of Sciences, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia; Fax: 7-383-2-33-36-77; E-mail: semenov@niboch.nsc.ru.

DOI: 10.1081/NCN-200026024
1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

Apoptosis is the genetically conserved cell suicide program directed to eliminate ''unwanted'' cells and to maintain the functionality and dynamic stability of cell populations in multicellular organisms. Oligonucleosome fragmentation of nuclear DNA is the late stage hallmark of the apoptotic process. In human apoptotic cells DNA fragmentation catalyzed by DFF40 is primarily activated by caspase 3 through the proteolytic digestion of DFF45, which is an inhibitor of DFF40.[1,2]

The absence of caspase 3 in MCF-7 cells has led to the disability to activate oligonucleosomal DNA fragmentation under TNF-α induced cell death.[3] At the same time fragmentation takes place when apoptosis of MCF-7 was induced with protein kinase inhibitor—staurosporine or xenobiotics paclitaxel or PBOX-6.[4–6]

Recently we have found that human milk induced apoptotic and necrotic cell death of MCF-7 cells. Free fatty acids appeared to be the prominent cytotoxic component of milk. Both whole milk and polyunsaturated fatty acids induced apoptosis of MCF-7 cells accomplished with oligonucleosome fragmentation of nuclear DNA. It is well known that at a toxic level of fatty acids, the caspase cascade initiates with mitochondrion collapse and activation of caspase 9.[7] Therefore in contrast to TNF-alpha, fatty acids trigger a different entering point in the apoptotic cascade and recruit a different apoptotic mechanism.

The purpose of the study is the exploration of mechanism of apoptotic DNA fragmentation induced by fatty acids in casp3 deficient adenocarcinoma MCF-7 cells.

METHODS

Cell Culture and Induction of Apoptosis. MCF-7 cells (ACTC) were maintained in IMDM medium (Sigma), supplemented with fetal calf serum (10% w/v), L-glutamine (2 mM), streptomycin (50 mg/ml). For induction of apoptosis, cells were incubated for 24 h with 50 ng/ml tumor necrosis factor-a (IBAS Vector, Novosibirsk) plus 10 mg/ml cycloheximide (Sigma) or in the same medium supplemented with 500 mkg/ml palmitate Na.

Apoptosis Assays. Annexin V-FITC binding and propidium iodide (PI) staining (Pharmingen) were performed according to the recommended protocol and the unfixed cells were analyzed by fluorescent microscopy. Apoptotic cells were defined as PI negative (indicating an intact plasma membrane) and annexin V-FITC positive relative to cells incubated in the absence of apoptoic agents.

Laddering of nuclear DNA was assessed by the protocol of Kaufmann, S.H. et. al.[8] The microsomal and cytosolic fractions of apoptotic MCF-7 were obtained by method.[9]

RESULTS AND DISCUSSION

It is known that most of laboratory clones of MCF-7 cells lack 47 b.p. in exon 4 of unique casp3 gen located in chromosome 4. Due to the deletion exon 4 is skipped at

splicing. Mature MCF-7 casp3 mRNA has an unchanged 5′-UTR and several first codons but as a result of the deletion, frame shift led to the formation of several premature stop codons. Therefore MCF-7 cells lack of the major apoptotic executioner–caspase 3.[3]

In order to confirm the absence of caspase 3 in our MCF-7 clone we analyzed the length of DNA-fragments amplified with the primers flanking the deletion (Fig. 1). So as the single DNA fragment ~80 b.p. was amplified from MCF-7 nuclear DNA, therefore the clone we used has a deletion of ~50 b.p. in the gene of caspase 3.

TNF-alpha induced apoptosis of caspase 3 deficient MCF-7 cells proceeds without chromatin condensation and DNA fragmentation. In contrast, medium condensed with sodium palmitate induced apoptotic nuclear changes and oligonucleosomal DNA fragmentation (Fig. 2).

Palmitate but not TNF-alpha induced apoptosis of MCF-7 cells accompanied with activation of doublet of 40 and 50 kDa DNases. 40–50 kDa DNases appear in the nuclear and cytoplasmic fractions of dying cells (Fig. 3).

The nearest structural homologue of caspase 3 is caspase 7. Caspase 7 induces DFF40 activation in vitro.[10] It is known that apoptotically activated caspase 7 almost exclusively associates with the mitochondrial and microsomal fractions of dying cells.[11] The microsome fraction of palmitate treated MCF-7 cells activates the DFF40-like nuclease when incubated with placental chromatin (Fig. 4a). Moreover microsomes of apoptotic MCF-7 cells induce oligonucleosome fragmentation of chromatin in a cell free system (Fig. 4b). When the reactions proceed in the presence of caspase 3,7 inhibitor—Ac-DEVD-CHO, neither activation of DNase nor DNA fragmentation occurs

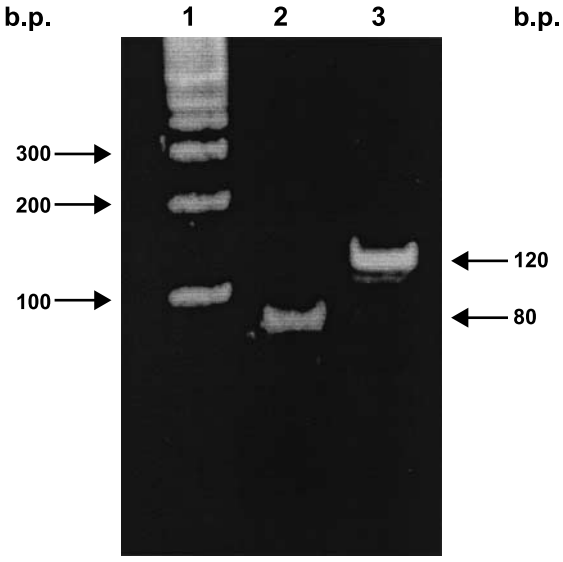

Figure 1. PCR—analysis of genome DNA from MCF-7 and human placenta with primers flanking the deletion in exon 4 of human casp3 gen. Amplified DNA was separated in 8% PAAG in native conditions and stained with EtBr.

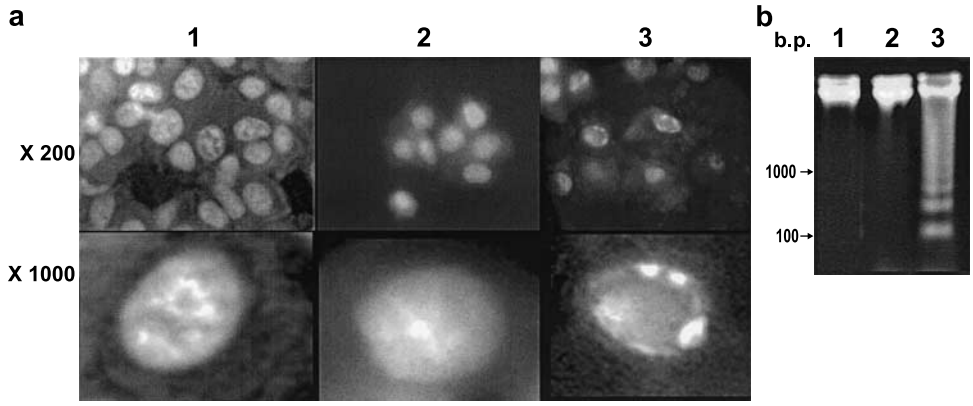

Figure 2. a) Nuclear changes of apoptotic MCF-7 treated with TNF-a (2), sodium palmitate (3) in comparison with untreated control (1). Fluorescent microphotography of paraformaldehyde fixed MCF-7 stained with propidium iodide. b) Fragmentation of nuclear DNA under apoptotic death of MCF-7 treated with TNF-alpha (2), palmitate Na (3) and DNA of untreated cells (1). DNA was extracted and analyzed by standard electrophoresis in 1.8% agarose gel.

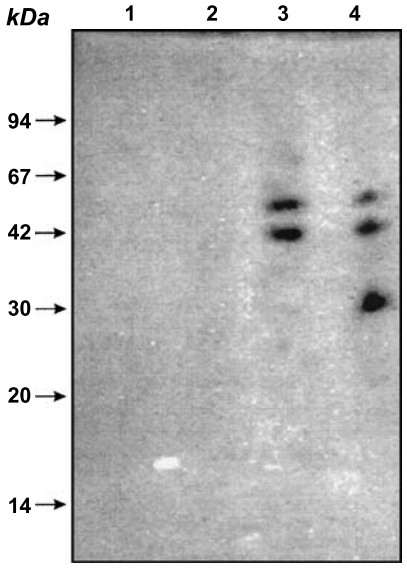

Figure 3. SDS-DNA-PAAG electrophoresis of nuclear (1–3) and cytoplasmic (4) proteins of MCF-7, treated with TNF-alpha (2), and palmitate Na (3, 4). Before separation gel was loaded with DNA. After electrophoresis gel was incubated in optimal conditions to restore DNAses, followed by EtBr DNA staining. Location of DNases visualized as unstained bands.

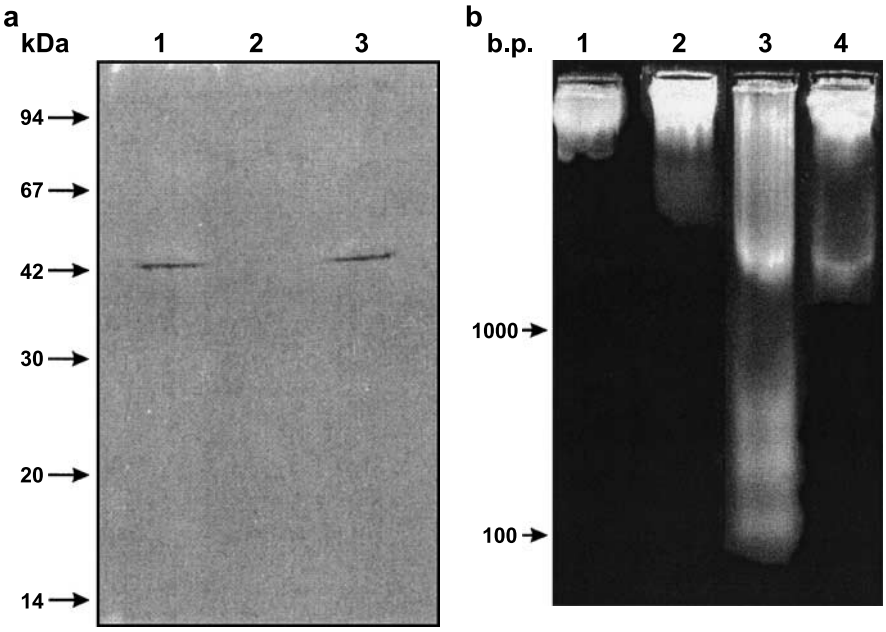

Figure 4. a) Activation of 40–50 kDa DNases inhibited by Ac-DEVD-CHO in cell-free system. Human placenta chromatin was incubated with microsome fraction of palmitate treated MCF-7 (1) in presence of DMSO solution of Ac-DEVD-CHO (2) or DMSO only (3). Activation of DNases was analyzed by SDS-DNA-PAAG electrophoresis followed by EtBr staining DNA. b) Fragmentation of chromatin DNA in presence of microsome fraction of apoptotic MCF-7. Placental chromatin (1) was incubated with microsomes of TNF-a (2) or palmitate treated MCF-7 (3, 4) in presence of Ac-DEVD-CHO (4) or without inhibitors (1–3). DNA in 1.8% agarose gel after electrophoresis was stained with EtBr.

(Fig. 4). Thus, it can be assumed that microsome associated caspase 7 is the most convenient candidate responsible for activation of DFF40-like DNAses in palmitate-induced apoptosis in the absence of caspase 3.

REFERENCES

1. Cryns, V.; Yuan, J. Proteases to die for. Genes Dev. **1998**, *12*, 1551–1570.
2. Cohen, G.M. Caspases: the executioners of apoptosis. Biochem J. **1997**, *326*, 1–16.
3. Janicke, R.U.; Sprengart, M.L.; Wati, M.R.; Porter, A.G. Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J. Biol. Chem. **1998**, *273*, 9357–9360.
4. McGee, M.M.; Hyland, E.; Campiani, G.; Ramunno, A.; Nacci, V.; Zisterer, D.M. Caspase-3 is not essential for DNA fragmentation in MCF-7 cells during apoptosis induced by the pyrrolo-1,5-benzoxazepine, PBOX-6. FEBS Lett. **2002**, *515*, 66–70.
5. Mooney, L.M.; Al-Sakkaf, K.A.; Brown, B.L.; Dobson, P.R. Apoptotic mechanisms in T47D and MCF-7 human breast cancer cells. Br. J. Cancer **2002**, *87*, 909–917.

6. 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.; Kaufmann, S.H. Lack of correlation between caspase activation and caspase activity assays in paclitaxel-treated MCF-7 breast cancer cells. J. Biol. Chem. **2002**, *277*, 804–815.
7. de Pablo, M.A.; Susin, S.A.; Jacotot, E.; Larochette, N.; Costantini, P.; Ravagnan, L.; Zamzami, N.; Kroemer, G. Palmitate induces apoptosis via a direct effect on mitochondria. Apoptosis **1999**, *4*, 81–87.
8. Kaufmann, S.H.; Mesner, P.W., Jr.; Samejima, K.; Tone, S.; Earnshaw, W.C. Detection of DNA cleavage in apoptotic cells. Methods Enzymol. **2000**, *322*, 3–15.
9. Rickwood, D.; Messent, A.; Patel, D.; Hinton, R.H.; Mullock, B.M. Isolation of subcellular fragments. In *Subcellular Fractionation—A Practical Approach*; Graham, J.M., Rickwood, D., Eds.; Oxford University Press: Oxford, UK, 1997; 75–76.
10. Chang, H.Y.; Yang, X. Proteases for cell suicide: functions and regulation of caspases. Microbiol. Mol. Biol. Rev. **2000**, *64*, 821–846.
11. Chandler, J.M.; Cohen, G.M.; MacFarlane, M. Different subcellular distribution of caspase-3 and caspase-7 following Fas-induced apoptosis in mouse liver. J. Biol. Chem. **1998**, *273*, 10815–10818.

Received December 2, 2003
Revised April 23, 2004