NuMA and nuclear lamins behave differently in Fas-mediated apoptosis

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Summary

NuMA is a nuclear matrix protein that has an essential function in the organization of the mitotic spindle. Here we have studied the fate of NuMA in Fas-treated apoptotic Jurkat T and HeLa cells. We show that in both cell lines NuMA is an early target protein for caspases and that NuMA is cleaved coincidently with poly(ADP-ribose) polymerase-1 (PARP-1) and nuclear lamin B. NuMA is cleaved differently in Jurkat T and HeLa cells, suggesting that different sets of caspases are activated in these cell lines. The normal diffuse intranuclear distribution of NuMA changed during apoptosis: first NuMA condensed, then concentrated in the center of the nucleus and finally encircled the nuclear fragments within the apoptotic bodies. NuMA seems to be preferentially cleaved by caspase-3 in vivo since it was not cleaved in staurosporine-treated caspase-3-null MCF-7 breast cancer cells. The cleavage of NuMA, lamin B and PARP-1 was inhibited in the presence of three different caspase inhibitors: z-DEVD-FMK, z-VEID-FMK and z-IETD-FMK. Furthermore, in the presence of caspase inhibitors approximately 5-10% of the cells showed atypical apoptotic morphology. These cells had convoluted nuclei, altered chromatin structure and additionally, they were negative for NuMA and lamins. Since caspase-8, -3 and -7 were not activated and PARP was not cleaved in these cells as judged by western blotting and immunofluorescence studies, it is likely that this is an atypical form of programmed cell death owing to a proteinase(s) independent of caspases. These results characterize the role of NuMA in programmed cell death and suggest that cleavage of NuMA plays a role in apoptotic nuclear breakdown.

Key words: NuMA, Lamins, Apoptosis, Caspases, Caspase independent

Introduction

Apoptosis or programmed cell death shows major biochemical and morphological changes in a cell. These include activation of a subfamily of cysteine proteases known as caspases, chromatin cleavage and condensation, shrinking of the cell, blebbing of cellular membranes and finally formation of the apoptotic bodies. Specific cleavage of several target proteins such as poly(ADP-ribose) polymerase-1 (PARP-1), DNA fragmentation factor (ICAD/DFF-45), lamins, fodrin, gelsolin, topoisomerase I, vimentin and Rb has been documented (reviewed by Nicholson, 1997), which is thought to result in the characteristic morphological changes seen during apoptosis.

One of the proteins to be degraded during apoptosis is NuMA (nuclear mitotic apparatus protein), a 238 kDa protein that is a component of the nuclear matrix during interphase and that redistributes to the spindle poles in mitosis (Lyderson and Pettyjohn, 1980; Kallajoki et al., 1992). Several studies have shown that NuMA is essential for normal mitosis as an organizer of the mitotic spindle (Kallajoki et al., 1991; Kallajoki et al., 1993; Yang and Snyder, 1992; Compton and Cleveland, 1993; Gaglio et al., 1995; Gaglio et al., 1996; Merdes et al., 1996). In the mitotic spindle, NuMA interacts with the dynein-dynactin complex (Gaglio et al., 1995; Gaglio et al., 1996; Merdes et al., 1996), and it seems that NuMA and another noncentrosomal protein, the human homologue of the KIN C motor family (HSET), co-operate in association with dynein to anchor microtubule minus ends at spindle poles and to support chromosome movement (Gordon et al., 2001). Recently, NuMA has been shown to be a part of a microtubule aster-promoting activity (APA), a multi-protein complex that induces spindle formation in mitosis and is regulated by small GTPase Ran and importin β (Nachury et al., 2001; Wiese et al., 2001).

The primary function of NuMA during interphase is still unclear. The cDNA sequence of NuMA shows homology to some structural filament-forming proteins such as cytokeratins, nuclear lamins and myosin heavy chain (Compton et al., 1992; Yang et al., 1992). Overexpression studies have shown that overexpression of NuMA lacking the nuclear localization signal results in cytoplasmic aggregates composed of 5 nm NuMA filaments (Saredi et al., 1996; Gueth-Hallonet et al., 1998), whereas overexpression of full-length NuMA leads to a quasi-hexagonal lattice-like structure in the nucleus (Gueth-Hallonet et al., 1998). Harborth et al. have also shown that NuMA can self assemble into multiarm oligomers in vitro (Harborth et al., 1999). All these results suggest that NuMA can, at least under some circumstances, form ordered structures and that it might have a structural function in the interphase nucleus. More recently, this has been supported by RNA interference (RNAi) studies; silencing of NuMA gene results in an apoptotic phenotype in HeLa cells suggesting that NuMA is essential for these cells (Harborth et al., 2001).

Several studies have shown that NuMA is specifically degraded in early apoptotic cell death. Weaver et al. showed that NuMA is cleaved into approximately 200 and 48 kDa fragments in dexamethasone-treated thymocytes (Weaver et al., 1996). A ~180 kDa form of NuMA was described in HeLa cells treated with 5,6-dichloro-1-β-D-ribofuranosyl-benzimidatsole and in HL60 cells treated with camptothecin, staurosporine, cycloheximide and A23187 (Hsu and Yeh, 1996). Multiple cleavage products of NuMA have also been reported in Jurkat T cells during Fas-mediated apoptosis (Casiano et al., 1996; Greidinger et al., 1996). In hydroxyurea- and staurosporine-treated BHK cells, NuMA is cleaved between residues 1701 and 1725, releasing the C-terminal tail domain, which contains a functionally important nuclear localization signal (Gueth-Hallonet et al., 1997). Immunofluorescence analysis has shown that the normal diffuse distribution of NuMA is changed during apoptosis and NuMA is excluded from the condensed chromatin (Gueth-Hallonet et al., 1997). Hirata et al. have further shown, using isolated nuclei from HeLa cells and recombinant caspases (Hirata et al., 1998), that at least caspase-3, -4, -6 and -7 degrade NuMA in vitro. Indeed, the cleavage of NuMA can be inhibited with several protease inhibitors including VEID-CHO, DMQD-CHO (Hirata et al., 1998) and TPCK but not with Ac-YVAD-cmk, TLCK or E-64 (Gueth-Hallonet et al., 1997). In addition to caspases, granzyme B has been shown to cleave NuMA (Andrade et al., 1998).

Another apoptotic nuclear target is nuclear lamina, the structure underlying the inner nuclear membrane and supporting the nuclear architecture. It is composed of four different intermediate filament proteins: lamins A, B1, B2 and C. Lamins B1 and B2 are encoded by two different genes (LMNB1 and LMNB2), and they are expressed in all mammalian somatic cells. Lamins A and C are generated from the LMNA gene by alternative splicing and are absent from some cell types (Guilly et al., 1987; Stewart and Burke, 1987; Röber et al., 1989). Lamins were among the first apoptotic target proteins identified, and the cleavage of lamins has been suggested to play a key role in the breakdown of nuclear structure (Lazebnik et al., 1995). It seems that lamin B is cleaved by caspase-3 (Slee et al., 2001) and lamin A and lamin C are cleaved by caspase-6 (Orth et al., 1996; Takahashi et al., 1996). Similar to NuMA, Granzymes have been recently shown to degrade lamins independently of caspases. Both granzyme A and B cleave lamin B, whereas granzyme A but not granzyme B cleaves lamins A and C (Zhang et al., 2001).

In the present study, we studied further the morphological and biochemical changes in NuMA during Fas-receptor-mediated apoptosis especially in relation to other nuclear structures and apoptotic target proteins including nuclear lamins and PARP-1. We describe the changes in the distribution of NuMA and lamins and show that degradation of NuMA is an early process preferentially due to caspase-3 activity since the cleavage is inhibited in the presence of certain caspase inhibitors and NuMA is not cleaved in caspase-3-null MCF-7 human breast cancer cells.

Materials and Methods

Cell culture

HeLa SS6 cells (American Type Culture Collection, a gift from John Eriksson, Turku Centre for Biotechnology, Turku, Finland) and an estrogen-dependent strain of MCF-7 human breast cancer cells [gift from C. K. Osborne, University of Texas (Osborne et al., 1987)] were cultured in DMEM. Jurkat T cells (American Type Culture Collection, a gift from John Eriksson) were cultured in RPMI 1640. Medium was supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. In addition, the medium of MCF-7 cells was supplemented with 10 nM β-estradiol (Sigma Chemical Co., St Louis, MO). HeLa and MCF-7 cells were cultured in 92 mm Petri dishes and divided twice a week to a concentration of ~10^6 cells/dish. Jurkat T cells were cultured in 30 ml bottles and divided three times a week to the concentration of ~10^6 cells/bottle.

Reagents

To induce apoptosis, Jurkat T cells were treated with 100 ng/ml of an agonistic anti-human Fas receptor IgM antibody (CH-11, MBL Medical & Biological Laboratories, Nagoya, Japan). To sensitize HeLa cells for Fas-mediated apoptosis, cells were treated with 100 ng/ml of Fas antibody in the presence of 30 μM MAPK-kinase inhibitor PD 98059 (Calbiochem, La Jolla, CA) as described previoulsy (Holmstrom et al., 1999). To induce apoptosis, MCF-7 cells were treated with 2 μM staurosporine (Sigma). Benzyloxi-carbonyl-Asp(Ome)–Glu(Ome)–Val-Asp(Ome) fluorometylketone (z-DEVD-FMK), Benzyloxicarbonyl-Val–Glu(Ome)–Ile-Asp(Ome)-fluorometylketone (z-VEID-FMK) and Benzyloxicarbonyl-Ile–Glu(Ome)–Thr-Asp(Ome)-fluorometylketone (z-IETD-FMK) were obtained from R&D Systems (Abingdon, UK) and dissolved into DMSO. They were added into cell culture coincidently with other reagents and diluted as shown in Results.

Immunofluorescence microscopy

For immunofluorescence microscopy HeLa and MCF-7 cells were grown on 12 mm glass coverslips. At the time intervals indicated in the results section, cells were washed once with PBS (145 mM NaCl, 7.5 mM Na_2HPO_4, 2.8 mM NaH_2PO_4) before fixing. For Jurkat T cells, 100-200 μl of cell suspension was placed on a silanized microscope slide for 30 minutes to attach the cells. Additional medium was then removed with filter paper. All samples were fixed in 3.7% formaldehyde in PBS for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes and washed with PBS. The coverslips were treated with 5% normal goat serum (Dako Immunochemicals, Klostrup, Denmark) in PBS or 5% BSA in PBS (when rabbit anti-goat secondary antibody was used) for 30 minutes, washed three times with PBS and incubated with primary antibody/antibodies for 2 hours at room temperature. Mouse monoclonal NuMA antibody [SPN-3 clone (Kallajoki et al., 1991; Kallajoki et al., 1993)] was used as undiluted culture supernatant and goat polyclonal lamin B antibody (Santa Cruz Biotechnology, CA), mouse monoclonal lamin A/C antibody (Novocastra Laboratories, Newcastle upon Tyne, UK), rabbit polyclonal PARP-1 p85 fragment antibody (Promega, Madison, WI) and cleaved caspase-3 (Asp 175) antibody (Cell Signaling Technology, Beverly, MA) were diluted 1:10, 1:20, 1:50 and 1:25 into 1% BSA in PBS, respectively. Coverslips were washed three times with PBS and then incubated with either FITC-conjugated goat anti-mouse IgG (Cappel Laboratories, Couchranville, PA, USA) and TRITC-conjugated goat anti-rabbit IgG (Zymed Laboratories, San Francisco, CA, USA) or FITC-conjugated rabbit anti-mouse IgG (Zymed) and TRITC-conjugated rabbit anti-goat IgG (Zymed) secondary antibodies for 1-1.5 hours at room temperature. After washing three times with PBS, samples were stained for DNA with Hoechst 33258 (1 μg/ml in 25% ethanol/75% PBS) for 5 minutes and embedded in Mowiol 4.88 (Hoechst AG, Frankfurt, Germany). TUNEL assays were performed using the DeadEnd Fluorometric TUNEL System (Promega). In brief, cells grown on coverslips were first fixed and permeabilized as described

above. Samples were pre-equilibrated with equilibrate buffer for 10 minutes at room temperature and then incubated with buffer containing equilibrating buffer, nucleotide mix and TdT enzyme for 1 hour at 37°C protected from light. The reaction was terminated by immersing the samples in 2×SSC (1×SSC; 0.15 M NaCl, 0.015 M trisodium citrate) for 15 minutes at room temperature. Samples were washed three times in PBS for 5 minutes and finally stained for DNA with Hoechst 33258. All samples were analyzed using Olympus BX 50 fluorescence microscope (Olympus Optical Co. LTD, Tokyo, Japan) and AnalySIS software (Soft Imaging Systems) or Leica confocal scanning laser microscope. To determine the amounts of apoptotic, atypical apoptotic and NuMA-negative cells on the coverslips, 800-1200 cells from at least eight randomly selected areas were counted for each sample. The mean values and the standard deviations were determined after three parallel experiments.

Electrophoresis and immunoblotting

HeLa and MCF-7 cells grown on 55 mm petri dishes were first scraped into the medium. Cell suspensions were then centrifuged at 1000 ***g*** for 5 minutes, washed with PBS and finally pelleted at 12,000 ***g*** for 5 minutes. Cell numbers were counted with a haemocytometer. Cell pellets were resuspended directly into hot SDS-PAGE electrophoresis sample buffer at a concentration equivalent to 10^7 cells/ml and sonicated for 5 seconds. Samples were loaded on 5% polyacrylamide gels for NuMA, on 5% or 10% gels for PARP-1, on 10% gels for lamins and on 10%, 12% or 14% gels for caspases. Two parallel gels were run: one for Coomassie brilliant blue staining to control equal loading and another for immunoblotting. Proteins were transferred electrophoretically to nitrocellulose (Schleicher & Schuell, Dassel, Germany) in a buffer containing 25 mM Tris, 192 mM glycine, 0.05% SDS and 10% methanol at 300 mA constant current for 1.5 hours. The transfer was controlled by Ponseau red staining. The filters were preincubated in 4% bovine serum albumin (BSA) in 0.2% Tween 20 in TBS (Tris-buffered saline: 20 mM Tris-HCl, pH 7.4, 0.15 mM NaCl) overnight and incubated with NuMA antibody (SPN-3), lamin A/C antibody (Novocastra), lamin B antibody (Santa Cruz Biotechnology), mouse monoclonal PARP-1 antibody (clone C-2-10, Sigma), rabbit polyclonal caspase-8 antibody (NeoMarkers, Fremont, CA, USA), rabbit polyclonal caspase-3 antibody (BD Pharmingen) or mouse monoclonal caspase-7 antibody (BD Pharmingen) diluted in 1% BSA, 0.2% Tween 20 in TBS for 2-3 hours at room temperature. The filters were washed three times with 0.2% Tween 20 in TBS and incubated for 1-1.5 hours at room temperature with peroxidase-labeled sheep anti-mouse IgG (Amersham, Buckinghamshire, UK), donkey anti-rabbit IgG (Amersham) or rabbit anti-goat IgG (Zymed) diluted 1:1500-10000 in 1% BSA, 0.2% Tween 20 in TBS. After three washes with 0.2% Tween 20 in TBS the immunoreactivity was detected by using enhanced chemiluminescence reaction (ECL Western blotting detection system, Amersham). When incubated with another primary antibody, filters were first washed with 0.2% Tween 20 in TBS, then incubated with +50°C stripping buffer (2% SDS, 100 mM β-mercaptoethanol, 63 mM Tris, pH 6.8) for 1 hour and washed four times with 0.2% Tween 20 in TBS.

Results

NuMA is cleaved in early phases in apoptotic Jurkat T cells

Although NuMA is degraded in several models of apoptosis, no closer morphological or time-schedule analysis has been done. To study these aspects in detail, Fas-receptor-mediated apoptosis was induced in two different cell lines. First, the well characterized Fas-mediated Jurkat T cell model was used. In control cells NuMA antibody showed normal diffuse or slightly granular staining in the nucleus excluding nucleoli, whereas lamin B antibody stained the nuclear lamina (Fig. 1A-E). In Fas-treated Jurkat cells, normal nuclear morphology was observed after 30 minutes (data not shown) but already 60 minutes after the induction few cells with early morphological changes of apoptosis were seen. These cells had shrunk and DNA staining showed that these cells had partly condensed chromatin typical of early apoptotic cells. Normal diffuse distribution of NuMA had condensed, whereas lamin B staining had got wrinkled (data not shown; Fig. 1F-J). After 120 minutes different phases of apoptosis including apoptotic

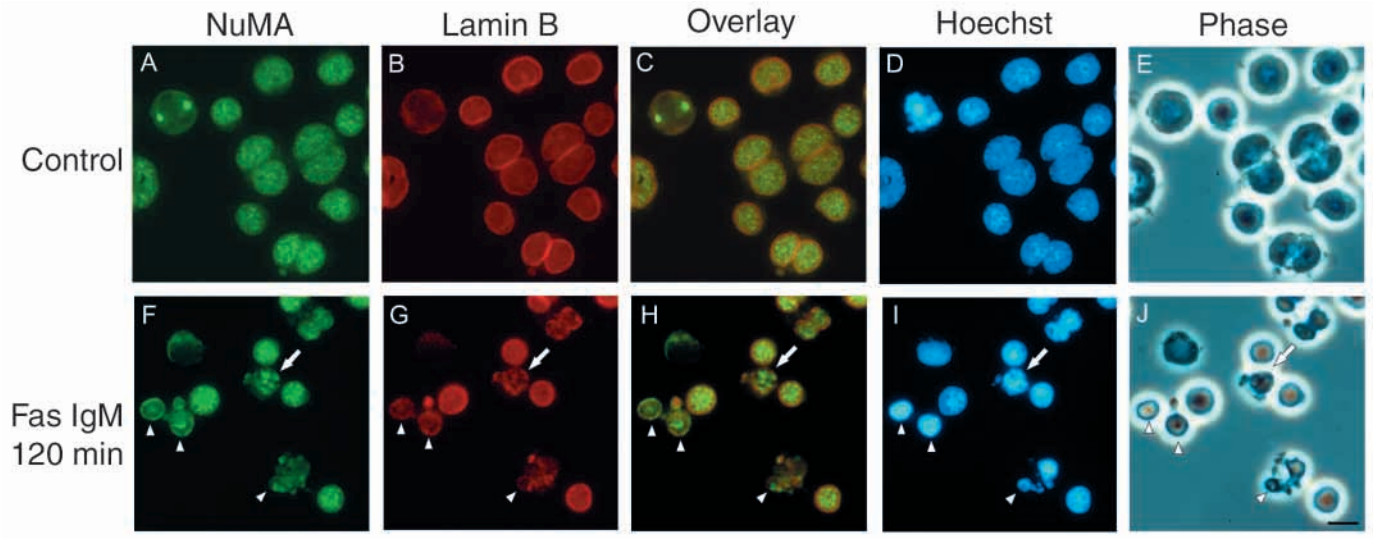

Fig. 1. Immunofluorescence microscopy of cultured Jurkat T cells during Fas-mediated apoptosis. Cells grown in suspension were untreated (A-E) or treated (F-J) with Fas antibody for 120 minutes, fixed and double-stained with NuMA (A and F) and lamin B (B and G) primary antibodies and FITC-conjugated rabbit-anti-mouse (A and F) and TRITC-conjugated rabbit-anti-goat (B and G) secondary antibodies. Samples were counterstained for DNA with Hoechst (D and I). Note the uneven condensed distribution of NuMA and wrinkled distribution of lamin B in early apoptotic cell (arrow) and the presence of NuMA and lamin B around fragmented nuclei in apoptotic bodies (arrowheads). Bar, 5 μm.

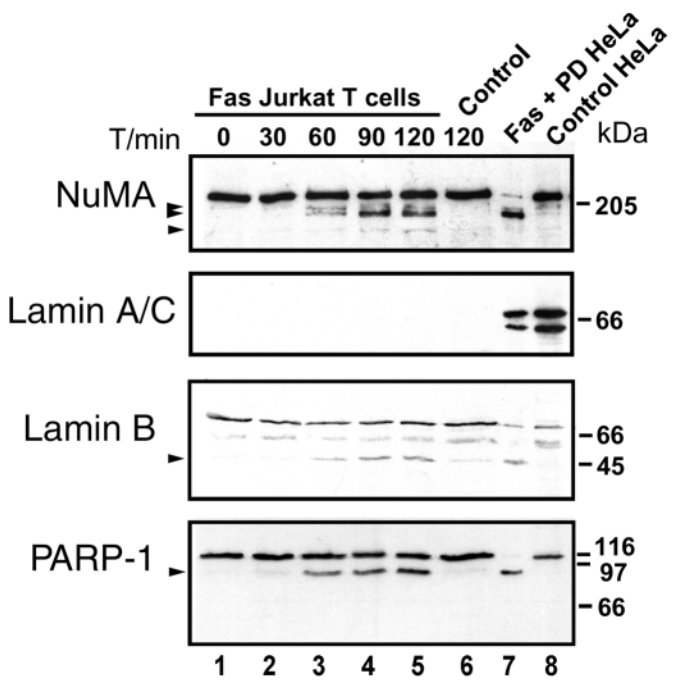

Fig. 2. The cleavage of NuMA, lamin B and poly(ADP-ribose) polymerase-1 (PARP-1) during Fas-mediated apoptosis in Jurkat T cells. Jurkat T cells were treated with Fas antibody and prepared for SDS-PAGE at different time points as indicated. Identically loaded gels were run and immunoblotted with NuMA, lamin A/C, lamin B or PARP-1 antibody. Arrowheads indicate the cleavage products of NuMA, lamin B and PARP-1. Lamin A/C antibody does not detect lamin A or C in Jurkat T cells. Fas-antibody- and PD 98059-treated and untreated HeLa cells were used as controls (both 36 hours, see later).

bodies were seen (Figs. 1F-J). Approximately 26% of cells were clearly apoptotic as judged by DNA staining in immunofluorescence microscopy. However, irresponsive cells with normal nuclear morphology and few mitotic cells were also seen. In apoptotic cells, both NuMA and lamin B encircled the fragmented nuclei within the apoptotic bodies when they were present (Fig. 1F-H).

To analyze the biochemical processing of NuMA during apoptosis and to compare it to the other known apoptotic target proteins, western blotting was used (Fig. 2). NuMA antibody detected one approximately 240 kDa protein in control cells. In apoptotic cells, NuMA was cleaved into two forms of approximately 180 and 190 kDa after 60 minutes. The latter is likely to represent an intermediate cleavage product of NuMA, since the 180 kDa fragment became more evident at later time points. In addition, a weaker ~160 kDa form of NuMA was observed after 90 and 120 minutes. When compared to other apoptotic target proteins, the cleavage of NuMA is an early process, which takes place simultaneously with the cleavage of lamin B and poly(ADP-ribose) polymerase-1 (PARP-1), a well characterized early apoptotic target protein cleaved by caspases-3 and -7 (Tewari et al., 1995; Germain et al., 1999; Slee et al., 2001). The lamin A/C antibody detected neither lamin A nor lamin C in Jurkat T cells, although both were detected in control HeLa cells.

Changes in NuMA and nuclear lamins in apoptotic HeLa cells

As the small size of Jurkat T cells sets limits to closer morphological analysis we continued with HeLa cells sensitized to Fas-mediated apoptosis with mitogen-activated protein kinase (MAPK) kinase inhibitor PD 98059 [a specific

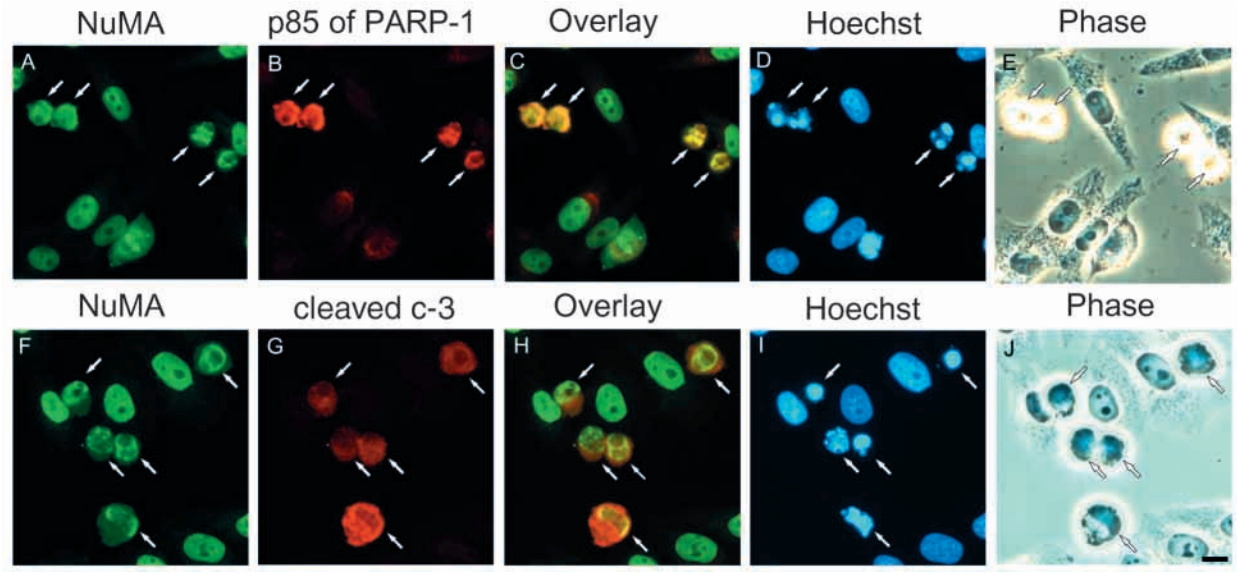

Fig. 3. Localization of NuMA, p85 fragment of PARP-1 and cleaved caspase-3 in cultured HeLa cells during Fas-mediated apoptosis. Cells grown on glass coverslips were treated with Fas antibody and PD 98059 for 16 hours and double-stained with NuMA (A) and the p85 fragment of PARP-1 (B) or NuMA (F) and cleaved caspase-3 (G) antibodies. FITC-conjugated goat-anti-mouse (A and F) and TRITC-conjugated goat-anti-rabbit (B and G) secondary antibodies were used. Samples were counterstained for DNA with Hoechst (D and I). In apoptotic cells, NuMA is condensed or localized outside the fragmented nuclei within the apoptotic bodies (A and F, arrows). Note that these cells are positive for cleaved p85 fragment of PARP-1 and cleaved caspase-3 (B and G). Bar, 10 μm.

inhibitor of the EKR1/2 pathway by inhibiting MKK1 (Alessi et al., 1995)] as described previously (Holmstrom et al., 1999). The distribution of NuMA was compared to changes in lamin B, lamins A/C and to DNA stain. When treated with 100 ng/ml of a Fas receptor antibody in the presence of 30 μM MAPK-kinase inhibitor, early morphological signs of apoptosis were noticed using phase contrast microscopy 8-12 hours after the induction of apoptosis. After 24 hours approximately 40-50% of the cells were detached into the culture medium. These cells were apoptotic as judged by immunofluorescence microscopy (data not shown). On glass coverslips, normal cells, early phases of apoptosis and cells with typical apoptotic bodies were seen when stained for DNA (Fig. 3D,I). Approximately 30% of adherent cells were apoptotic (see later Fig. 8). NuMA was found to encircle the fragmented nuclei in the apoptotic bodies (Fig. 3A,F). To ensure that the morphological changes described were truly apoptotic, samples were double stained with NuMA antibody and antibodies detecting either the cleaved p85 fragment of PARP-1 or the cleaved caspase-3 (Asp175). Fig. 3A-E shows that the cells in which NuMA is excluded from the condensed chromatin stain with the p85 fragment specific PARP-1 antibody, whereas normal cells do not. An antibody against cleaved caspase-3 first showed cytoplasmic bright spots in the cells with shrunken nuclei and

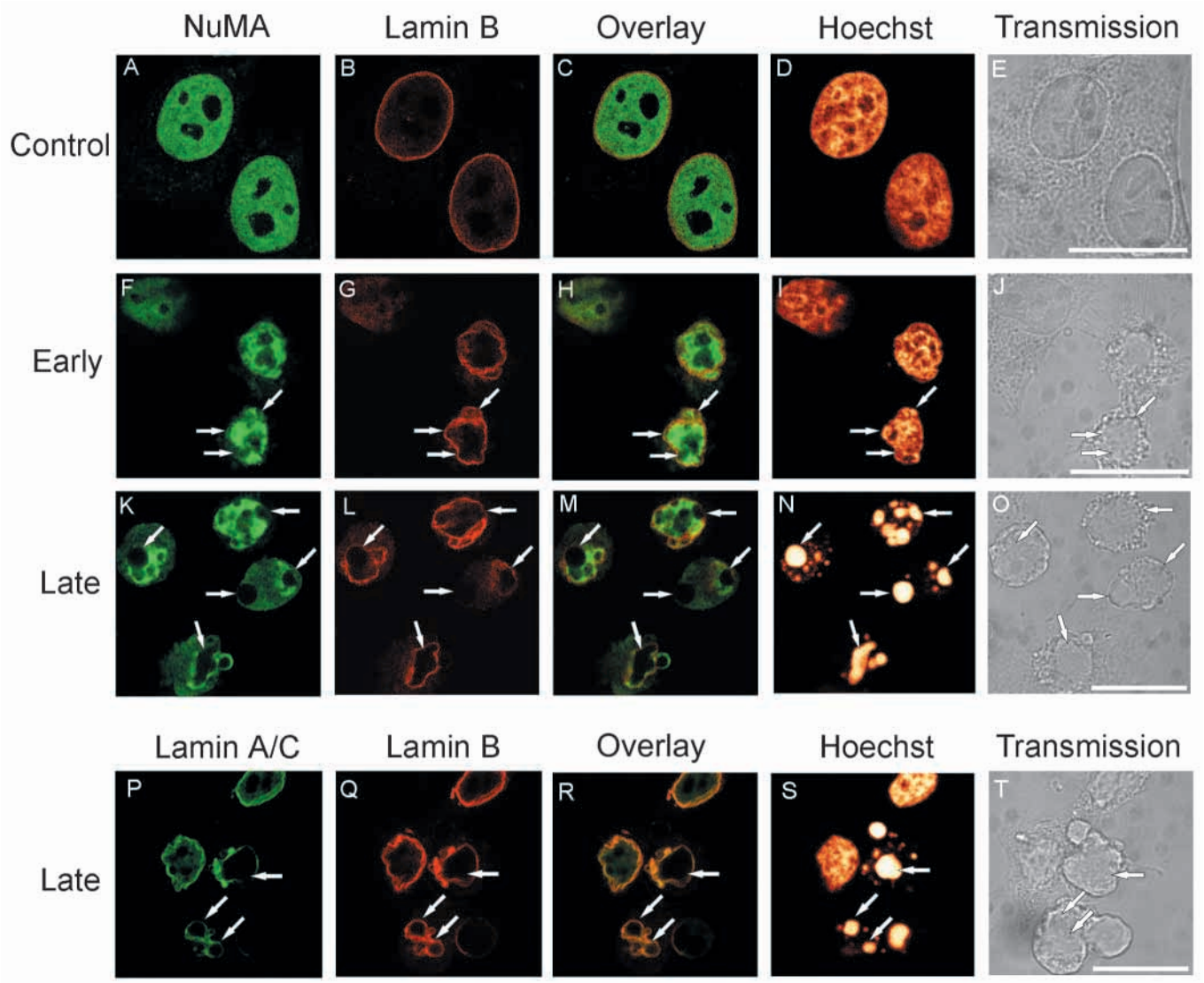

Fig. 4. Confocal microscope analysis of cultured HeLa cells during Fas-mediated apoptosis. Cells grown on glass coverslips were untreated (A-E) or treated with Fas antibody and PD 98059 for 24 hours (F-T) and prepared for immunofluorescence microscopy. Samples were double-stained with NuMA (A, F and K) and lamin B (B, G and L) or lamin A/C (P) and lamin B (Q) antibodies. FITC-conjugated rabbit-anti-mouse (A, F, K and P) and TRITC-conjugated rabbit-anti-goat (B, G, L and Q) secondary antibodies were used. Samples were counterstained for DNA with Hoechst (D, I, N and S; Hoechst stain is shown in glow color). In the beginning of apoptosis NuMA condenses in the center of the nucleus (F) and excludes the condensed chromatin in the nuclear periphery (arrows). Lamin B shows a folded staining pattern (G). Note that lamin B staining shows the upper surface of the nuclear lamina in a normal interphase cell. At the end of apoptosis, NuMA partially encircles the NuMA-negative nuclear fragments (arrows) within apoptotic bodies (K). Lamin B remains around NuMA and the fragmented nuclei (L). Lamin A/C colocalizes with lamin B (P-R). Transmission views show typical apoptotic features including shrinking of the cell and nucleus, cytoplasmic blebbing, detaching of the cells and finally formation of the apoptotic bodies (E, J, O and T). Bar 10 μm.

partially condensed NuMA and chromatin (data not shown). At the later stage, cleaved caspase-3 located diffusively in the whole cell excluding the nuclear fragments (Fig. 3F-J).

When viewed with confocal microscopy, NuMA staining was granular excluding nucleoli in normal interphase cells (Fig. 4A). In early apoptotic cells NuMA started to concentrate in the center of the nucleus and chromatin condensed close to the nuclear rim (Fig. 4F-J). At the end of apoptosis, NuMA clearly redistributed around the NuMA-negative fragmented nuclei in the apoptotic bodies (Fig. 4K-O). Lamin B staining showed that the normal circle-like staining pattern changes into a folded distribution in the beginning of apoptosis (Fig. 4G). Later, Lamin B condensed and seemed to encircle NuMA and the fragmented nuclei (Fig. 4L). The double staining with both NuMA and lamin B antibodies revealed that these proteins do not usually colocalize (Fig. 4C,H,M). Lamins A and C seemed to behave in a same way as lamin B (Fig. 4P), and in double staining these proteins colocalized in apoptotic nuclei (Fig. 4R).

NuMA is cleaved differently in Fas-treated HeLa cells

For western blot analysis both detached and adherent cells were harvested. Fig. 5 shows that NuMA is cleaved into an approximately 180 kDa product in apoptotic HeLa cells. In addition, the ~190 kDa intermediate fragment was observed in the beginning of apoptosis but neither ~160 kDa fragment nor other smaller fragments were detected even 36 hours after induction of apoptosis although ~100% of the cells were apoptotic and no full-length NuMA was detected in western blots. The cleavage of NuMA was an early process beginning 8 hours after induction of apoptosis, and it was highly coincident with the cleavage of PARP-1 and lamin B into typical apoptotic 85 kDa and 46kDa fragments, respectively. Surprisingly, the antibody detecting both 70 kDa lamin A and 60 kDa lamin C detected no major degradation products. Only a minimal amount of approximately 50 kDa product was seen

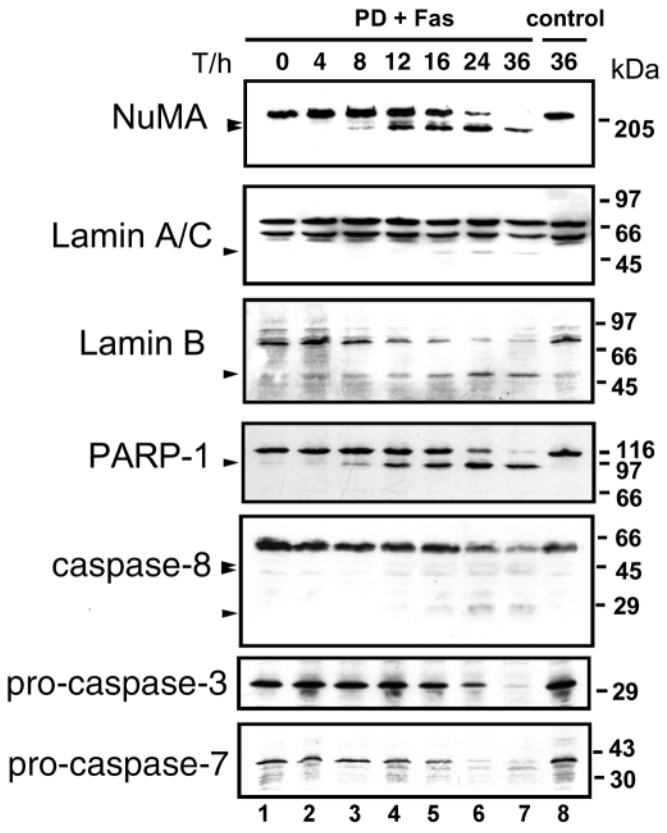

Fig. 5. The cleavage of NuMA, lamins A/C, lamin B, PARP-1 and pro-caspase-8, -3 and -7 during Fas-mediated apoptosis in HeLa cells. Cells grown on a monolayer were treated with Fas antibody and PD 98059 and prepared for SDS-PAGE at different time points as indicated. Identically loaded gels were run and immunoblotted with NuMA, lamin A/C, lamin B, PARP-1, caspase-8, caspase-3 or caspase-7 antibody. Note the gradual cleavage of NuMA and PARP-1 after 8 hours and lamin B after 8-12 hours. Lamins A and C are only partially cleaved during apoptosis. The amounts of procaspase-8, -3 and -7 are diminished and the cleaved 42/44 and 25 kDa fragments of caspase-8 are increased during apoptosis.

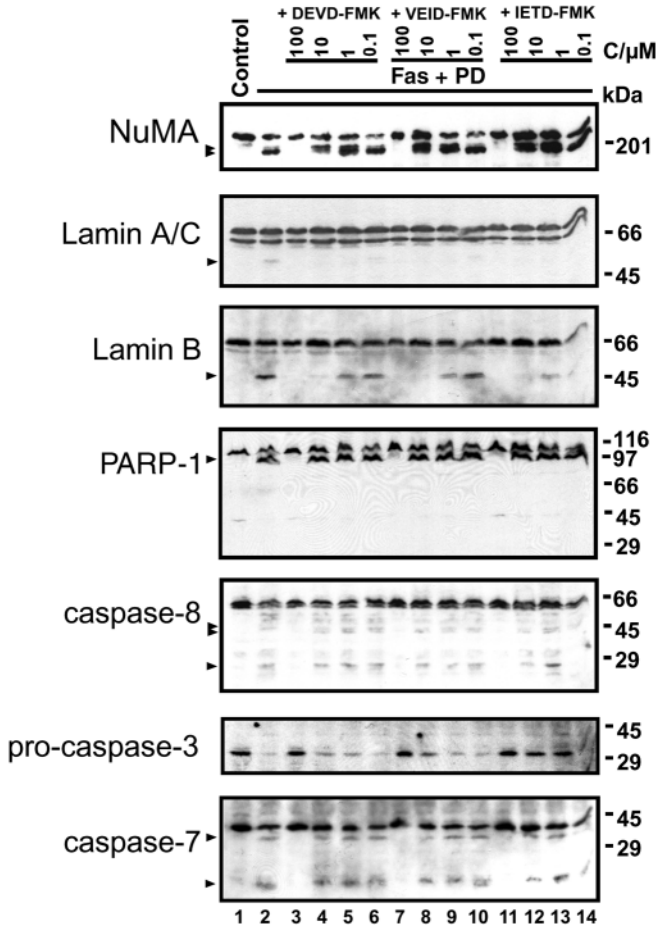

Fig. 6. The cleavage of NuMA, lamin A/C, lamin B, PARP-1 and procaspase-8, -3 and -7 is inhibited in the presence of z-DEVD-FMK, z-VEID-FMK or z-IETD-FMK. HeLa cells grown on a monolayer were treated with Fas antibody and PD 98059 for 24 hours in the presence or absence of 100, 10, 1 or 0.1 μM z-DEVD-FMK, z-VEID-FMK or z-IETD-FMK. Cells were prepared for SDS-PAGE and immunoblotted with NuMA, lamin A/C, lamin B, PARP-1, caspase-8, caspase-3 or caspase-7 antibody. Note, that z-DEVD-FMK, z-VEID-FMK and z-IETD-FMK inhibit the cleavage of NuMA, lamins, PARP-1 and pro-caspase-8, -3 and -7 identically and dose dependently.

after 16 hours. The amounts of lamins A and C were not diminished, suggesting that lamins A and C were not significantly cleaved during apoptosis. To ensure that caspases are truly activated in Fas-ligated HeLa cells, immunoblotting with caspase-8, -3 and -7 antibodies was done. Fig. 5 shows that the amounts of 55/57 kDa proform of caspase-8, 32 kDa proform of caspase-3 and 35 kDa proform of caspase-7 were diminished, apparently owing to their activation. In addition, the cleaved 42/44 kDa and ~25 kDa fragments of caspase-8 were detected after 16 hours.

The cleavage of NuMA is inhibited in the presence of caspase inhibitors

To find out whether degradation of NuMA is really due to caspase activity and to find out which caspase cleaves NuMA in vivo we induced apoptosis in the presence of different caspase inhibitors. Three peptide-based, cell-permeable and irreversible caspase inhibitors were used: z-DEVD-FMK, z-VEID-FMK and z-IETD-FMK. These are suggested to inhibit caspases-3, -6 and -8, respectively. They all act as substrates for the caspases, bind to the active site, form irreversible bond with the enzyme and block the caspase from further action. Fig. 6 shows that when used in a 100 μM concentration for 24 hours, all these inhibitors managed to inhibit the cleavage of NuMA, lamins, PARP-1 and proforms of caspase-8, -3 and -7. Actually all these inhibitors had an identical dose-dependent effect when compared to each other. In a 10 μM concentration the cleavage of NuMA and PARP-1 was only partly inhibited whereas the cleavage of lamins was still effectively inhibited. The 1 μM concentration had only little and the 0.1 μM concentration practically no effect on the cleavage of the proteins tested.

To examine the effects of the caspase inhibitors on the morphology of the cells treated, immunofluorescence analysis was done. When treated with Fas antibody and PD in the presence of 100 μM z-DEVD-FMK, z-VEID-FMK or z-IETD-FMK for 24 hours all cells were adherent and only single apoptotic cells were seen. Interestingly, a population of cells with altered apoptotic nuclear morphology was also seen (Fig. 7). With Hoechst staining these cells had heavily convoluted nuclei with slightly condensed chromatin structure (Fig. 7B,D). Moreover, these nuclei seemed to be negative or weakly positive when stained with NuMA (Fig. 7A), lamin A/C (Fig. 7C) or lamin B (data not shown) antibodies. In the presence of 30 μM z-DEVD-FMK even more cells with atypical and typical apoptotic nuclei existed when incubated for 24 hours. In addition, cells with an abnormal distribution of nuclear NuMA were found (Fig. 7E). The normal diffuse distribution of NuMA had changed and NuMA had condensed into multiple areas in the nucleus. Hoechst stain shows that these cells do not have shrunken nuclei, the nucleoli are still visible but the nuclei are slightly convoluted and they have altered chromatin structure (Fig. 7F).

We next determined the amounts of apoptotic, atypical apoptotic and NuMA-negative cells on the coverslips in different samples. Fig. 8 shows that in the presence of Fas antibody, PD and 100 μM z-DEVD-FMK, z-VEID-FMK or z-IETD-FMK approximately 0-2% and 4-10% of the cells showed apoptotic and atypical apoptotic nuclei, respectively, whereas in the presence of only Fas antibody and PD, 25-35%

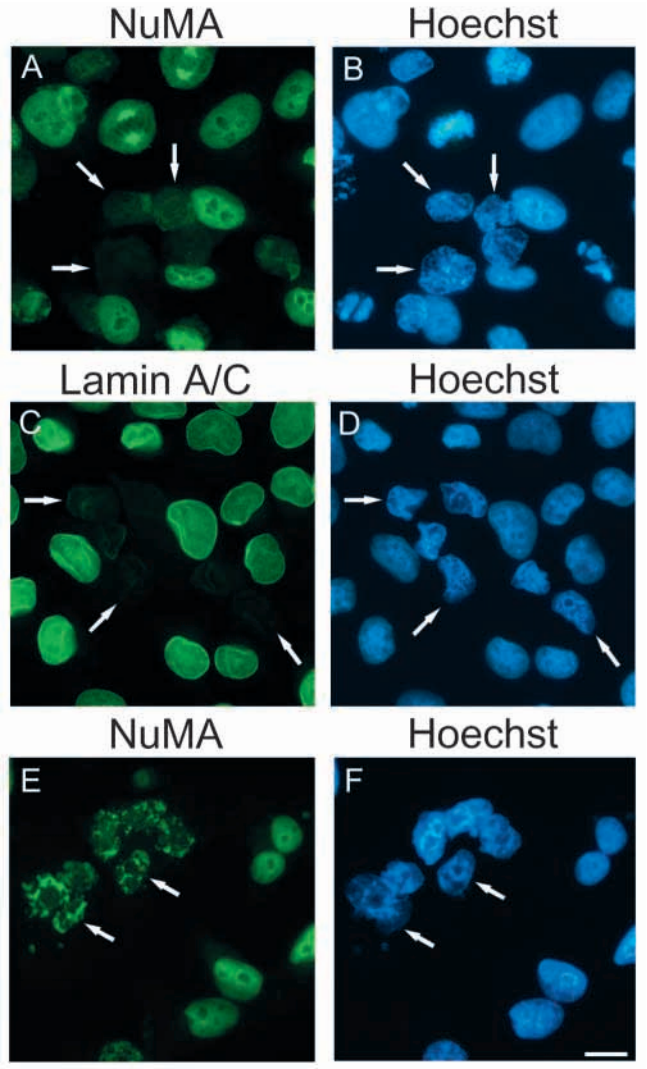

Fig. 7. Immunofluorescence microscopy of Fas-treated HeLa cells in the presence of z-DEVD-FMK. Cells grown on glass coverslips were treated with Fas antibody and PD 98059 for 24 hours in the presence of 100 μM (A-D) or 30 μM (E and F) z-DEVD-FMK and stained with NuMA (A and E) or lamin A/C primary antibody (C) and with FITC-conjugated goat-anti-mouse secondary antibody. Samples were counterstained for DNA with Hoechst (B, C and F). Note the atypical apoptotic cells (arrows) in the presence of 100 μM z-DEVD-FMK, in which chromatin is partially condenced and the nuclear outer border is convoluted. These cells are negative for NuMA (A) and lamin A/C (C). In the presence of 30 μM z-DEVD-FMK, chromatin is slightly condenced in atypical apoptotic cells (F, arrows) and the nuclei have lost their normal round shape. NuMA staining shows highly irregular distribution inside the nucleus (E). Bar 10 μm.

of the cells were apoptotic but no atypical apoptotic cells were found. The amount of NuMA-negative cells also correlated with the amount of atypical apoptotic cells as shown in Fig. 8. To find out whether this atypical apoptotic morphology was due to the effect of caspase inhibitors only, HeLa cells were treated with 100 μM z-DEVD-FMK for 24 hours. However, neither apoptotic nor atypical apoptotic cells were found in the samples.

To investigate whether atypical apoptotic morphology

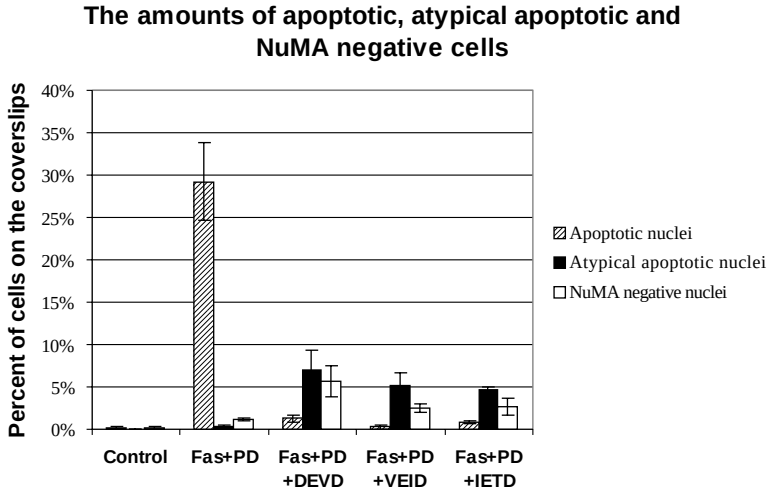

Fig. 8. The amount of apoptotic, atypical apoptotic and NuMA negative nuclei in the presence of caspase inhibitors. HeLa cells grown on glass coverslips were treated with Fas antibody and PD 98059 for 24 hours in the presence or absence of 100 μM z-DEVD-FMK, z-VEID-FMK or z-IETD-FMK and processed for immunofluorescence microscopy analyses. 800-1200 adherent cells in randomly selected areas were counted for each coverslip. The data represent mean values and standard deviations from three separate experiments. Note the correlation between the numbers of atypical apoptotic and NuMA negative nuclei.

described above was due to caspase activation, cells treated with Fas antibody and PD in the presence of 30 μM z-DEVD-FMK for 24 hours were stained with cleaved caspase-3 and p85 fragment of PARP-1 antibodies. Since the atypical apoptotic cells also showed partial chromatin condensation, we performed a TUNEL assay to reveal possible DNA cleavage into oligonucleosomal 180-200 bp fragments. Fig. 9 shows that typical apoptotic cells stain with both antibodies and incorporate fluorescein-12-dUTP, whereas atypical apoptotic cells do not. According to this data, it seems that caspase-3 is not activated in atypical apoptotic cells but rather this is an early morphological change owing to upstream caspases or another proteinases independent of effector caspases.

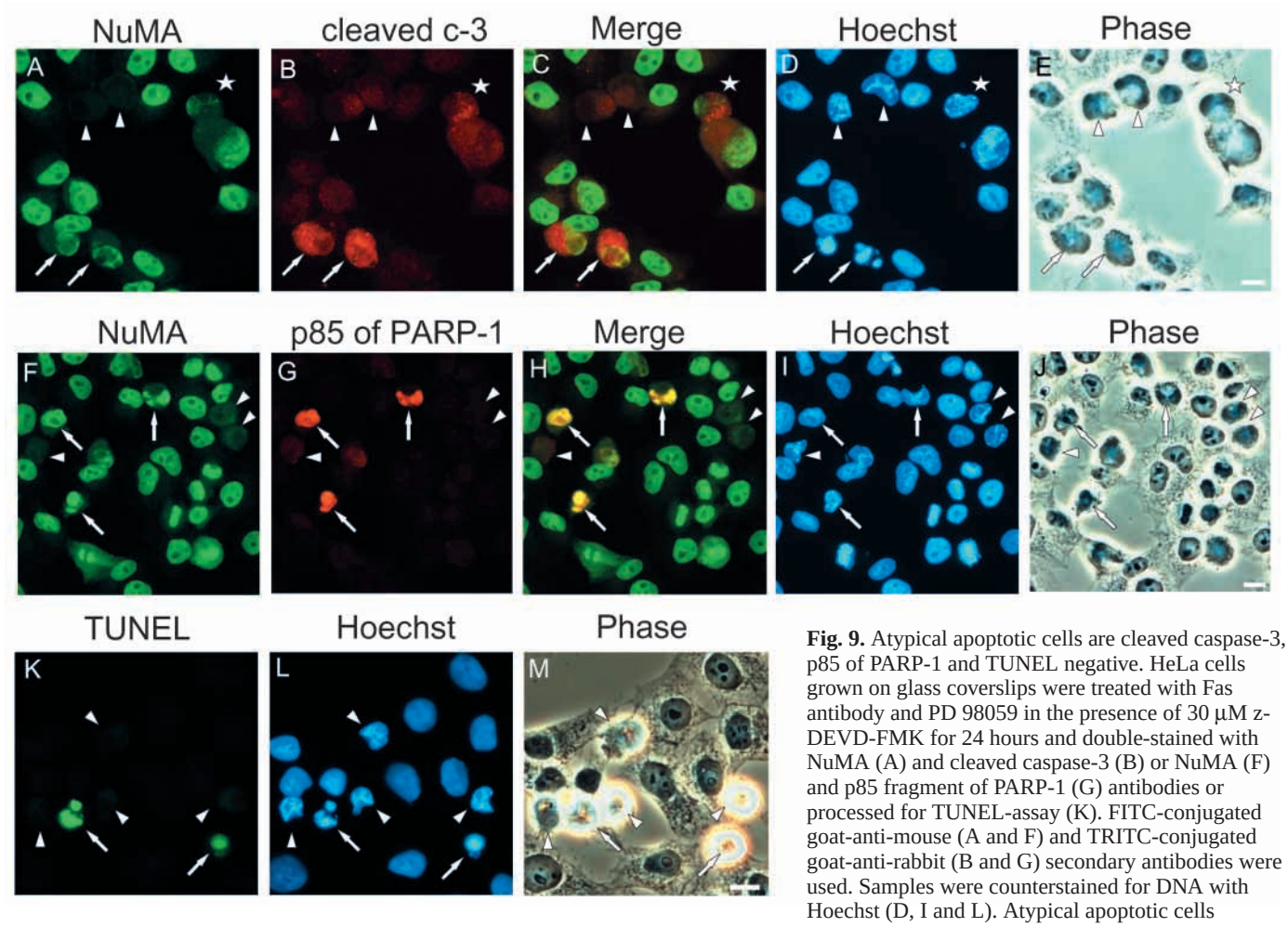

Fig. 9. Atypical apoptotic cells are cleaved caspase-3, p85 of PARP-1 and TUNEL negative. HeLa cells grown on glass coverslips were treated with Fas antibody and PD 98059 in the presence of 30 μM z-DEVD-FMK for 24 hours and double-stained with NuMA (A) and cleaved caspase-3 (B) or NuMA (F) and p85 fragment of PARP-1 (G) antibodies or processed for TUNEL-assay (K). FITC-conjugated goat-anti-mouse (A and F) and TRITC-conjugated goat-anti-rabbit (B and G) secondary antibodies were used. Samples were counterstained for DNA with Hoechst (D, I and L). Atypical apoptotic cells (arrowheads) are NuMA and TUNEL negative and do not stain with cleaved caspase-3 or p85 fragment of PARP-1 antibodies. Typical apoptotic cells (arrows) stain with all studied antibodies and are TUNEL positive. Note the single cell (star, A-E) which stains weakly for NuMA and cleaved caspase-3 and shows abnormal chromatin structure. The phase contrast view shows that both typical and atypical apoptotic cells have shrunken and partially detached. Bar 10 μm.

NuMA or lamins are not cleaved in staurosporine treated caspase-3-null MCF-7 cells

Since caspases-3 and -7 were the most probable candidates responsible for the cleavage of NuMA, we tested the fate of NuMA in MCF-7 human breast cancer cell line known to lack caspase-3 (Jänicke et al., 1998b). MCF-7 cells undergo apoptosis lacking several morphological features of apoptosis like cytoplasmic blebbing and formation of apoptotic bodies (e.g. Jänicke et al., 1998b). When treated with 2 μM staurosporine for 12 hours as described previously (Johnson et al., 2000), the whole cells and the nuclei shrank and a part of the cells detached (Fig. 10F-O). DNA staining showed shrunken nuclei and partially condensed chromatin (Fig. 10I,N). In a few cells chromatin was even concentrated to the nuclear periphery (data not shown). When stained with NuMA antibody (Fig. 10F,K), all cells were NuMA positive but NuMA was only seldom separated from the chromatin. Cytoplasmic blebbing and formation of apoptotic bodies were not observed. In western blotting, NuMA antibody detected the full-length NuMA but, interestingly, no typical apoptotic cleavage products after 12 hours of incubation with 2 μM staurosporine (Fig. 11). Similarly, when immunoblotted with the lamin A/C and lamin B antibodies, no cleavage products were noticed, suggesting that lamins were not cleaved. Interestingly, PARP-1 antibody showed the appearance of apoptotic p85 fragment in staurosporine-treated cells. This was supported by immunofluorescence studies; when stained with a specific p85 fragment of PARP-1 antibody, a population of apoptotic cells were positive with this antibody (Fig. 10K-O). Previously caspase-7 has been shown to cleave PARP-1 in apoptotic MCF-7 cells (Germain et al., 1999). In agreement with this, immunoblotting with caspase-7 antibody showed that the amount of procaspase-7 was diminished presumably owing to its cleavage/activation. A partial cleavage of 55/57 kDa procaspase-8 into 42/44 kDa fragments was also detected in staurosporine-treated cells but the active fragment was not detected. The 32kDa procaspase-3 was not detected in MCF-7 cells although it was detected in control Jurkat T cells (data not shown). In summary, these results further suggest that although both caspase-3 and -7 cleave NuMA in vitro, caspase-3 is the main effector caspase to cleave NuMA in vivo.

Discussion

Several proteases cleave NuMA in apoptosis

Previous studies have shown that NuMA is degraded during apoptosis induced with various chemical agents (Hsu and Yeh, 1996; Weaver et al., 1996; Gueth-Hallonet et al., 1997; Sodja

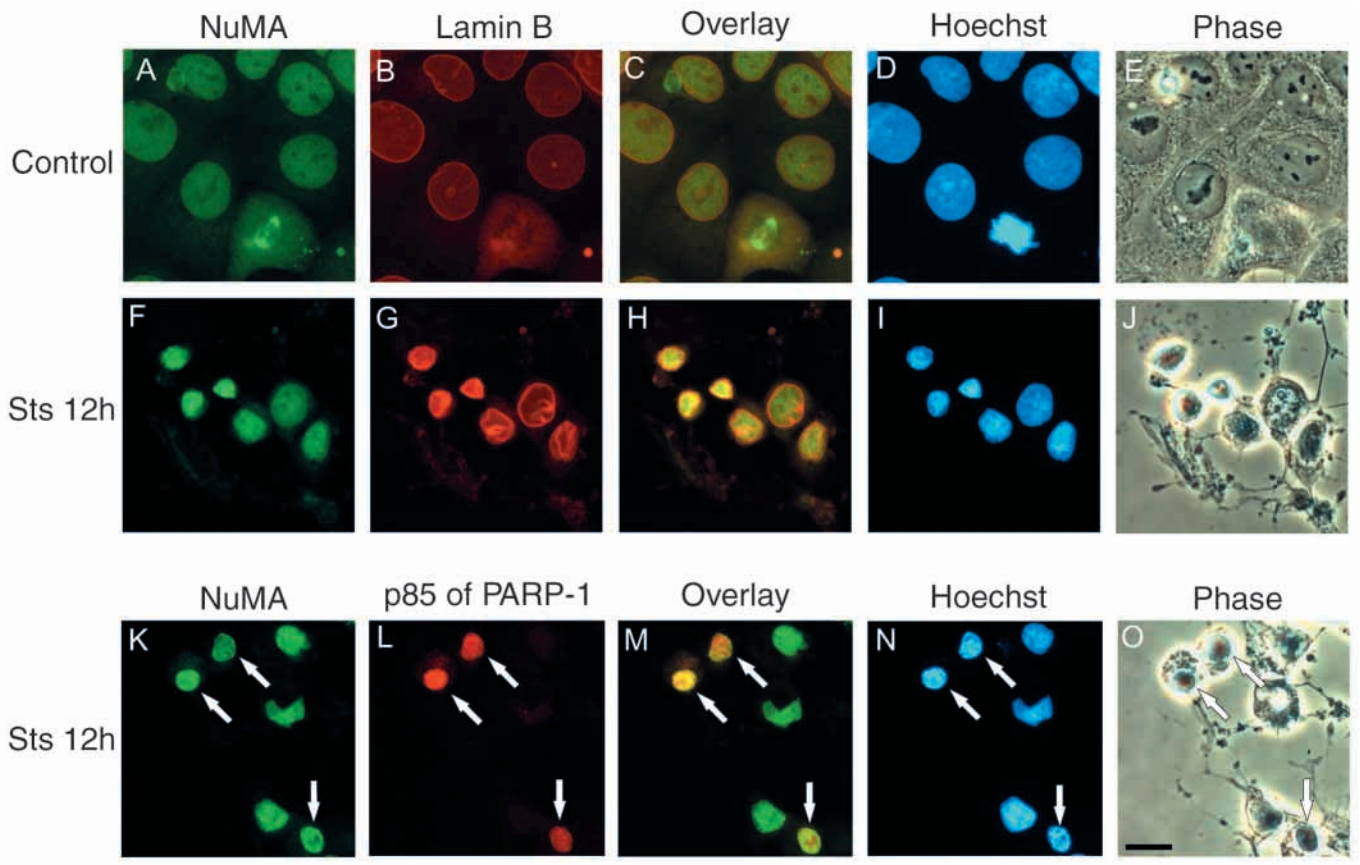

Fig. 10. Immunofluorescence microscopy of MCF-7 cells during staurosporine-induced apoptosis. Cells grown on the coverslips were untreated (A-E) or treated (F-O) with 2 μM staurosporine for 12 hours, fixed and double-stained with NuMA (A and F) and lamin B (B and G) or NuMA (K) and p85 fragment of PARP-1 (L) primary antibodies and FITC-conjugated rabbit-anti-mouse (A and F) and TRITC-conjugated rabbit-anti-goat (B and G) or FITC-conjugated goat-anti-mouse (K) and TRITC-conjugated goat-anti-rabbit (L) secondary antibodies. Samples were counterstained for DNA with Hoechst (D, I and N). Note the partially condensed distribution of NuMA and wrinkled distribution of lamin B in shrunken apoptotic cells. Hoechst stain shows that DNA is partially condensed. These cells are p85 fragment of PARP-1 positive (arrows). Bar, 10 μm.

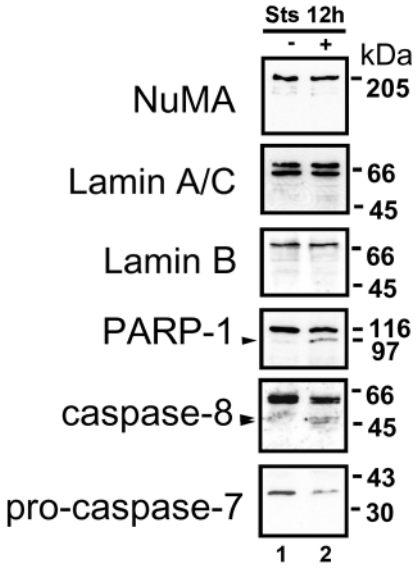

Fig. 11. NuMA and lamins are not degraded in MCF-7 cells during staurosporine-induced apoptosis. Cells grown on a monolayer were untreated (lane 1) or treated (lane 2) with 2 μM staurosporine for 12 hours and prepared for SDS-PAGE. Identically loaded gels were run and immunoblotted with NuMA, lamin A/C, lamin B, PARP-1, caspase-8 or caspase-7 antibody. Note the partial cleavage of PARP-1, procaspase-8 and disappearance of pro-caspase-7 in staurosporine-treated cells. NuMA and lamins are not cleaved during the apoptosis.

et al., 1998) or by incubating Jurkat T cells with Fas (CD95/APO-1) receptor ligating antibody (Casiano et al., 1996; Greidinger et al., 1996; Hirata et al., 1998). The present study supports this data. The incubation with Fas receptor antibody resulted in the cleavage of NuMA into ~180 and ~190 kDa fragments in both Jurkat T and HeLa cells (Figs 2 and 5). The 190 kDa fragment was further proteolyzed into an 180 kDa fragment (Fig. 5). In addition, the ~160 kDa fragment was detected in Jurkat T cells after 90 and 120 minutes (Fig. 2), which is consistent with earlier studies (Casiano et al., 1996; Hirata et al., 1998). In HeLa cells the 160 kDa fragment was not detected, which suggests that different proteolytic enzymes are activated in HeLa and Jurkat T cell lines. In the presence of caspase inhibitors (Fig. 6) and in apoptotic caspase-3-deficient MCF-7 cells (Fig. 11) NuMA was not cleaved, suggesting that NuMA is cleaved by caspase-3 and/or another protease downstream caspase-3 in vivo. Furthermore, the 160 kDa fragment appeared later than 180 and 190 kDa fragments, which suggests that the protease cleaving the 160 kDa fragment is downstream of those resulting in the 180 and 190 kDa fragments. Hirata et al. showed that although recombinant caspase-3, -4, -6 and -7 all cleave recombinant NuMA or NuMA in isolated HeLa nuclei in vitro (Hirata et al., 1998), only recombinant caspase-6 results in the production of a 160 kDa NuMA fragment. Therefore, it seems probable that caspase-3 produces the 180/190 kDa fragments and caspase-6 the 160 kDa fragment. The absence of the 160 kDa fragment in Fas-treated HeLa cells suggests that caspase-6 is not activated. This conclusion is also supported by the data that lamins A and C were not cleaved in these cells (Fig. 5), since caspase-6 has been shown to cleave lamin A (Orth et al., 1996; Takahashi et al., 1996).

The role of caspase-4 remained unclear in this study but since caspase-4 is not among the effector caspases in Fas-mediated apoptosis it is unlikely that it would play a significant role in the cleavage of NuMA in Fas-treated Jurkat T or HeLa cells. Caspase-7, however, was activated (Fig. 5) and cleaves NuMA similarly to caspase-3 in vitro (Hirata et al., 1998). Thus, it is possible that both caspase-3 and -7 cleave NuMA in Fas-treated HeLa cells. In staurosporine-treated caspase-3-deficient MCF-7 cells pro-caspase-7 and -8 and PARP-1 were cleaved, whereas NuMA and lamins were not (Fig. 11). Indeed, there is evidence that caspase-7 is activated in staurosporine- and TNF/cycloheximide-treated MCF-7 cells (Germain et al., 1999), although this was not completely supported (Jänicke et al., 1998a). Taken together, these results indicate that caspase-3 is necessary for the cleavage of NuMA and lamin B in vivo, whereas PARP-1 can be cleaved also in the absence of caspase-3, probably by caspase-7.

The amino-acid sequence of NuMA does not contain any specific caspase cleavage site when scanned for enzymatic cleavage sites with Exspasy Peptidecutter (Swiss Institute of Bioinformatics, University of Geneva and University of Lausanne, Switzerland). According to the molecular weight of the fragments the predicted caspase cleavage site of NuMA is DSLD-L between residues 1726-1727 (Nicholson and Thornberry, 1997). Caspases-3 and -7 prefer this sequence (DxxD) in a target protein. However, Gueth-Hallonet et al. showed that at least one cleavage site is located between residues 1701-1725 (Gueth-Hallonet et al., 1997). This sequence contains three Asp residues at positions 1705, 1719 and 1723, all of which can serve as a potential cleavage site for caspases. In their studies, changing D to G at position 1705 did not prevent the cleavage of the molecule. To determine the specific cleavage site(s) of NuMA, N-terminal sequencing or mass spectrometry analysis of the cleavage fragments is required.

The changes in NuMA and lamins during apoptosis

The time schedule/kinetics of cleavage of NuMA compared to other nuclear caspase substrates examined revealed that in both cell lines NuMA was cleaved coincidently with PARP-1 and lamin B. In Jurkat T-cells this time point was 60 minutes, which is exactly the same as shown previously (Casiano et al., 1996). Greidinger et al. noticed a minimal cleavage of PARP-1 and NuMA already 30 and 50 minutes after induction of apoptosis, respectively (Greidinger et al., 1996). However, all these studies show that NuMA is among the first substrates cleaved in Fas-mediated apoptosis. In both cell lines studied, the amount of apoptotic cells correlated with the amount of cleaved NuMA in western analysis: in Jurkat T and HeLa cells ~26% and ~65% of all cells in the sample were apoptotic after 120 minutes and 24 hours, respectively. The immunofluorescence and confocal microscope analysis of apoptotic HeLa cells (Figs 3 and 4) revealed that the apoptotic breakdown of the nucleus is a rapid process since relatively few early apoptotic cells were found at a certain time point. The normal distribution of NuMA changed simultaneously with the chromatin condensation approximately 12-16 hours after the induction of apoptosis. On the other hand, a small amount of cleaved NuMA was detected by western blotting already after 8 hours. Therefore, it is possible that cleavage of NuMA detaches it from the

condensed chromatin. Since apoptotic Jurkat T and HeLa cells showed similar nuclear morphology in immunofluorescence analysis we propose that the cleavage of NuMA into an 160 kDa fragment does not play a significant role in apoptosis.

Our results concerning the fate of nuclear lamins during apoptosis showed that different caspases cleave lamin A/C and B, since lamin B but not lamin A/C was cleaved in Fas-treated HeLa cells. This is consistent with the earlier studies (Orth et al., 1996; Takahashi et al., 1996; Slee et al., 2001). Caspase-3 and -6 are the main candidates for cleaving lamin B and A/C, respectively (Orth et al., 1996; Slee et al., 2001). Our results support this. Interestingly, Fas-treated HeLa cells showed typical apoptotic features, although lamins A and C were not degraded. This shows clearly that cleavage of lamins A and C is not a prerequisite of the apoptotic breakdown of the nucleus. The fact that lamins A and C are not expressed in all nucleated cell types (Guilly et al., 1987; Stewart and Burke, 1987; Röber et al., 1989) favor the idea that lamin B but not lamins A and C is essential for nuclear structure. Indeed, in agreement with Slee et al. (Slee et al., 2001) we were not able to detect any lamin A/C in Jurkat T cells by immunoblotting, suggesting that this cell type lacks lamin A/C. Finally, recent RNA interference studies have confirmed this hypothesis: silencing of lamin B1 or B2 gene results in an apoptotic phenotype in HeLa cells, whereas silencing of the lamin A/C gene has no effect on the viability of the cells (Harborth et al., 2001).

The effects of caspase inhibitors

Previously, the inhibition of NuMA cleavage has been reported from studies using a few caspase inhibitors (Gueth-Hallonet et al., 1997; Hirata et al., 1998). In the present study, three different peptide-based caspase inhibitors, z-DEVD-FMK, z-VEID-FMK and z-IETD-FMK, were tested. When used in a concentration of 100 μM, all these inhibitors managed to inhibit the cleavage of NuMA, lamins, PARP and proforms of caspase-3, -6 and -8 either directly or by blocking the caspase pathway and activation of downstream effector caspases (Fig. 6). The latter is highly probable especially in the case of z-IETD-FMK since it inhibits caspase-8, which is the main initiator caspase in Fas-mediated apoptosis. Although the inhibitors are designed to be specific for a certain caspase they also inhibit other caspases especially when used in higher concentrations (e.g. Hirata et al., 1998). z-DEVD-FMK, for example, inhibits caspase-3 but also partly inhibits caspases-7 and -8 (Sigma, manufacturers' data). z-VEID-FMK, which inhibits caspase-6, was also able to abolish the cleavage of pro-caspase-8, although caspase-6 is activated downstream of caspase-8 in Fas-mediated apoptosis (Fig. 6). Therefore, it seems clear that these inhibitors serve as general caspase inhibitors by inhibiting all caspases in a 100 μM concentration. To find out the possible differences between the effects of the inhibitors, lower inhibitor concentrations were tested. However, the results were similar when used at the same concentration.

In the presence of caspase inhibitors a cell population with an altered nuclear morphology was observed (Figs 7 and 9). It is possible that in these cells the apoptotic shrinkage and the degradation of the nucleus is delayed and/or this morphology is due to another caspase-independent cell death pathway. The latter is supported by several studies and, indeed, it has recently been shown that the Fas receptor can initiate another caspase-8-independent cell death pathway in Jurkat T cells at least in the presence of pan-caspase inhibitor z-VAD-FMK (Holler et al., 2000). In the present study, the presence of z-DEVD-FMK, z-VEID-FMK and z-IETD-FMK resulted in a similar morphology (Figs 7 and 9). In western blot analysis we did not detect cleavage of any pro-caspase or target protein studied and atypical apoptotic cells did not stain with cleaved caspase-3 or p85 fragment of PARP-1 antibodies, suggesting that caspases were not activated. Moreover, these cells did not stain with NuMA or lamin antibodies, which is different from that seen in the nuclei of typical apoptotic cells. Cells were also TUNEL-negative, indicating that DNA was not cleaved into oligonucleosomal fragments, and ICAD/DFF-45 [inhibitor of caspase activated DNAse (Enari et al., 1998)] was not cleaved by caspase-3 in these cells. Therefore, we conclude that this change is truly an atypical feature of apoptosis or secondary necrosis, which takes place independently of caspases. A similar morphology was also seen in tumor necrosis factor (TNF)-induced WEHI-S fibrosarcoma cells (Foghsgaard et al., 2001). In their study, cathepsin B, a noncaspase proteinase, resulted in a cell death with apoptotic features in the presence of pan-caspase inhibitor. Whether or not cathepsin B played a role in atypical apoptotic morphology seen in the present study remained unclear.

Atypical apoptotic cells were usually NuMA, lamin A/C and lamin B negative or only small NuMA-containing particles were observed in the nucleus (Figs 7 and 9). However, in the presence of 100 μM caspase inhibitors, cleaved form of NuMA was not observed in western blots (Fig. 6), suggesting that NuMA is not cleaved in these cells. A few possibilities have to be discussed: firstly, it is possible that this cell population is too small to detect their cleaved NuMA but this is very unlikely since up to 10% of the cells showed this morphology. Secondly, it is possible that NuMA is cleaved in the area at the epitope recognized by the antibody (amino acids 255-267). Thirdly, it is possible that NuMA and lamins are not cleaved but degraded by another unspecific protease or set of proteases. Neither can we exclude the possibility that the negative phenotype is due to inaccessibility of the antibodies to the nuclear proteins in these circumstances. This seems unlikely because we did not find any problems in staining of the other cells in the same sample.

The significance of degradation of NuMA

Although NuMA has been used as a marker to indicate the breakdown of the nuclear matrix (Hirata et al., 1998; Sodja et al., 1998), the significance of the cleavage of NuMA still remains unclear. NuMA is a component of the nuclear matrix, which can form ordered structures in the interphase nucleus, and it binds to defined DNA sequences called matrix-associated regions (MARs) in vitro (Luderus et al., 1994). Thus, the degradation of NuMA could result in the breakdown of normal nuclear architecture. Granzyme B also cleaves NuMA directly with similar efficiency to caspase-3, which highlights the importance of degradation of NuMA in caspase-independent apoptotic cell death (Andrade, 1998). It is also known that NuMA is degraded in necrotic HL-60 cells (Bortul et al., 2001). By contrast, it seems that certain cell types lack NuMA, and NuMA is preferentially expressed in the nuclei of

proliferating cells (Merdes and Cleveland, 1998; Sanghavi et al., 1998; Taimen et al., 2000). This suggests that NuMA is not, at least in all cells, needed for the formation of the nuclear structure. Moreover, apoptotic human neutrophils lacking NuMA show caspase-3 activation and lamin B cleavage, which suggests that the cleavage products of NuMA are not required in neutrophil apoptosis (Sanghavi et al., 1998). If the early change of chromatin structure showed here in atypical apoptotic cells truly results in the disappearance of NuMA from the nucleus, it is clear that the cleavage of NuMA is not essentially needed for the structural breakdown of the cell nucleus.

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