Staurosporine treatment and serum starvation promote the cleavage of emerin in cultured mouse myoblasts: involvement of a caspase-dependent mechanism

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Abstract Emerin is a nuclear membrane-anchored protein which is absent or mutated in patients affected by Emery–Dreifuss muscular dystrophy. In this study, we induced apoptosis in cultured mouse myoblasts to evaluate emerin fate during the nuclear destabilization involved in programmed cell death. Emerin proteolysis was observed in myocytes during the apoptotic process. Myoblast apoptosis and emerin degradation were associated with chromatin compaction and detachment from the nuclear lamina, as detected by electron microscopy. In vivo specific inhibition of caspase 3 or caspase 6 activity completely abolished emerin proteolysis. These results show that the process of programmed cell death in muscle cells leads to emerin proteolysis, which appears to be related to caspase 6 activation and to cleavage of other nuclear envelope proteins, that share sequence homologies or functional features with emerin.

Key words: Emerin; Apoptosis; Myoblast; Nuclear envelope; Caspase 3; Caspase 6

1. Introduction

Features of programmed cell death consist of a very particular nuclear behavior involving chromatin progressive margination and compaction, frequently appearing as cup-shaped compact areas [1]. In the following stages, the original nucleus splits in a number of dense micronuclei, scattered throughout the cytoplasm [2]. The late stages, are characterized by the presence of a variable number of apoptotic bodies which undergo secondary necrosis or phagocytosis by neighboring cells [3,4]. The destruction of the structural organization of chromatin is secondary to DNA cleavage [5] and proteolysis of nuclear components which, in turn, are responsible for nuclear destabilization.

A number of nuclear membrane and nuclear lamina [6] components have been implicated in the apoptotic process, including lamina associated polypeptide 2β (LAP2β) [7] and α (LAP2α) [8], lamin B receptor (LBR) [9] and proteins of the nuclear pore complex [7,9]. Lamins [6,10,11], poly(ADP-ribose)polymerase (PARP) [12–14], topoisomerase I and II and a number of constitutive transcription factors [15] are also cleaved in apoptotic nuclei.

Emerin is a serine-rich nuclear membrane-anchored protein which shows a sequence similarity with LAP2 and MAN1 [16,17]. The protein is linked to the nuclear lamina [18] and interacts with lamin A/C [19–21]. Emerin functional interaction with lamin A/C has been demonstrated: (1) during mitosis, emerin is redistributed in the cytoplasm following the fate of lamins [22,23]; emerin and lamin A/C colocalize at the nuclear envelope, but do not colocalize after mitotic nuclear membrane breakdown [22]; (2) embryonic fibroblasts obtained from mice null for the lamin A/C gene show a displacement of emerin from the nuclear envelope and a relocalization of this protein to the cytoplasm [20]; (3) mutations in emerin or lamin A/C genes cause the same disease, the Emery–Dreifuss muscular dystrophy (EDMD) [24,25]. The clinical symptoms of EDMD are early contractures, muscle weakness and wasting and cardiomyopathic defects [26]. In most X-linked EDMD affected patients, emerin is absent from the nuclear membrane of any cell type.

While it is well known that lamin A/C is cleaved by caspase 6 during programmed cell death [6], the behavior of emerin in apoptotic nuclei has only been described in Jurkat T cells following anti-Fas antibody treatment [8]. In this experimental model emerin is not processed during apoptosis and the authors suggest a role for emerin in maintaining chromatin association with the nuclear envelope in apoptotic lymphoblastoid cells [8]. Since muscle is the affected tissue in the EDMD, it appears of great interest to investigate emerin fate during myoblast apoptosis. Previously, it has been reported that emerin expression is related to myogenic differentiation of cultured myoblasts [27], suggesting a role for this ubiquitous protein in the turn-over of muscle tissue. Programmed cell death occurs during in vitro myogenesis [28] and during embryonic muscle development [29]; indeed, myoblasts undergo programmed cell death during normal proliferation and differ-

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Abbreviations: LAP2, lamina associated polypeptide 2; LBR, lamin B receptor; PARP, poly(ADP-ribose)polymerase; EDMD, Emery–Dreifuss muscular dystrophy; FCS, fetal calf serum; TUNEL, terminal transferase-mediated dUTP nick end-labeling

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entiation, but after fusion, myotubes acquire resistance to apoptosis [30–32].

In this study, we analyzed emerin fate in proliferating mouse myoblasts and differentiating myotubes during the apoptotic breakdown of the nucleus. Induction of apoptosis by two different protocols allowed us to show that emerin proteolysis occurs in apoptotic myoblast nuclei and it is related to activation of caspase 6, the protease involved in lamin A/C cleavage.

2. Materials and methods

2.1. Cell culture

Mouse C2C12 myoblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal calf serum (FCS, Gibco BRL Life Technologies, Paisley, UK). As the cells approached confluence, the growth medium was replaced with differentiation medium (DMEM plus 2% horse serum) to obtain multinucleated myotubes. Apoptosis was induced by serum deprivation of the culture [31] for 24, 48 or 72 h or by treatment with 1 μM staurosporine [28]; both exponentially growing myoblasts and myotubes at day 5 of differentiation were treated and harvested as floating cells. Cells were in part employed for the biochemical analysis, while an aliquot of cells was used for DNA extraction and agarose gel electrophoresis. Laminin-coated slides were used to culture myoblasts to be analyzed by immunocytochemistry.

2.2. Incubation of cultures with caspase inhibitors

Caspase 3 inhibitor Ac-Asp-Glu-Val-L-aspartic acid aldehyde (Ac-DEVD-CHO) and caspase 6 inhibitor Ac-Val-Glu-Ile-aspartic acid aldheyde (Ac-VEID-CHO) were purchased from Calbiochem (La Jolla, CA, USA). Each inhibitor was added to myoblast cultures 2 h before staurosporine administration and treatment was applied for additional 8 h. Both inhibitors were used at 50 and 100 μM concentration [7].

2.3. DNA ladder analysis

Total DNA was purified from cell pellets obtained from either control or treated myoblasts after staurosporine administration or serum starvation for the specified time. A DNA extraction kit (Qiagen, Italy) was used according to the manufacturer's protocol and DNA concentration was determined by measuring the absorbance at 260 nm. Aliquots of 5 μg of DNA in TE buffer (0.1 M Tris–HCl, pH 8.0, 10 mM EDTA) were loaded for each sample on 2% agarose gel, subjected to electrophoresis at a constant current of 20 V, visualized with ethidium bromide (0.4 μg/ml) by a UV transilluminator (302 nm) and the gel was photographed with a Polaroid camera.

2.4. Immunoblotting

Myoblasts and myotubes were lysed in a buffer containing Tris–HCl pH 7.4, 1% Nonidet P-40, 10 mM 2-mercaptoethanol, and protease inhibitors (leupeptin 2 μg/ml, aprotinin 2 μg/ml, PMSF 1 mM). Aliquots corresponding to 90 μg proteins were subjected to 15% SDS–PAGE, transferred onto a nitrocellulose membrane and Western blotted with the specified antibodies. All antibodies were diluted in phosphate-buffered saline (PBS)–Tween 20 at the following dilutions: anti-emerin, monoclonal (Novocastra, UK), 1:250; anti-emerin (C-terminus), polyclonal (Santa Cruz, CA, USA), 1:100; anti-emerin polyclonal [33], 1:500; anti-lamin A/C, polyclonal (Santa Cruz), 1:1500; anti-PARP, polyclonal (Cell Signaling Technologies, MA, USA), 1:300. Secondary antibodies were diluted as suggested by the manufacturer. Experiments were run on three different cultures for each point. Immunoblotted bands were visualized by enhanced chemiluminescence (Amersham International, UK).

2.5. Immunocytochemistry

Expression of emerin was evaluated in myoblast cultures treated with staurosporine for 8 h, using monoclonal anti-emerin antibody. Lamin A/C distribution was detected by a polyclonal antibody. Fixed cells were permeabilized with 0.15% Triton X-100 and incubated in PBS–4% bovine serum albumin pH 7.4 to block non-specific binding. Primary antibodies were incubated at 4°C overnight, while secondary antibodies were used at room temperature for 30–60 min. The following dilutions were employed: anti-emerin, monoclonal, 1:20; anti-lamin A/C, 1:100; anti-mouse IgG Cy3-conjugated (Sigma, MO, USA), 1:10 000 and anti-goat IgG fluorescein isothiocyanate-conjugated (Dako), 1:100. Slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI, 0.1 μg/ml) (Sigma). Control slides were performed in the absence of the primary antibodies.

2.6. Electron microscopy

Cell pellets from control myoblasts and from 18 h staurosporine-treated myoblasts were fixed with 2.5% glutaraldehyde −0.1 M phosphate buffer pH 7.6 for 1 h at room temperature. After post-fixation with 1% OsO_4 in veronal buffer for 1 h, pellets were dehydrated in an ethanol series and embedded in Epon resin. Formvar carbon-coated grids were used as thin sections support. Thin sections stained with uranyl acetate and lead citrate were observed with a Philips EM 400 transmission electron microscope.

2.7. DNA breaks detection by in situ end-labeling and cell viability analysis

The percentage of apoptotic nuclei in myoblast cultures transfected with the full-length emerin cDNA or with the empty vector was evaluated after staurosporine treatment. To label apoptotic nuclei, the 3′-OH end of DNA fragments were visualized by the method of terminal transferase-mediated dUTP nick end-labeling (TUNEL) using the Apoptosis Detection System fluorescence kit (Promega, WI, USA) according to the manufacturer's instructions.

3. Results

Emerin expression and proteolysis were studied in apoptotic nuclei of C2C12 myoblasts and myotubes. Both staurosporine treatment and serum starvation of cultures were effective in inducing myoblast apoptosis as shown by the analysis of lamin A/C and PARP cleavage.

3.1. Kinetics of emerin proteolysis

The time-course of emerin expression in C2C12 apoptotic myoblasts is reported in Fig. 1; staurosporine-treated myoblasts showed the same kinetics of emerin proteolysis as observed for the serum deprivation alone, but with a faster time-course. Emerin proteolysis was detectable in staurosporine-treated myoblasts (Fig. 1A) at the time-point 4 h. In serum-starved cells (Fig. 1B) the proteolysis of emerin started within 24 h of apoptosis induction. The emerin 34 kDa band was strongly reduced at 18 h of staurosporine treatment as well as at 72 h of serum starvation. After 30 h of staurosporine treatment or 96 h of serum starvation emerin was hardly detectable by Western blot analysis, possibly because secondary degradation of protein occurred at these late stages of the apoptotic process.

A cleavage peptide of about 20 kDa was detected in all the apoptotic samples by Western blot analysis performed with a polyclonal antibody raised against the C-terminus of mouse emerin. At the time-point 18 h the native 34 kDa band was hardly detectable in staurosporine-treated myoblasts, while the low molecular weight fragment was sharply labeled.

Cells still adherent to the plastic substrate at any time-point, and after induction of apoptosis using either experimental protocol, showed a slightly reduced emerin band of 34 kDa, as expected for pre-apoptotic cells (not shown) [28].

3.2. Emerin fate in apoptotic myotubes

Emerin proteolysis was also studied in apoptotic myotubes (Fig. 2A); staurosporine treatment or 48 h serum deprivation were effective in inducing a strong reduction of emerin amount in differentiated cells. To rule out the possibility

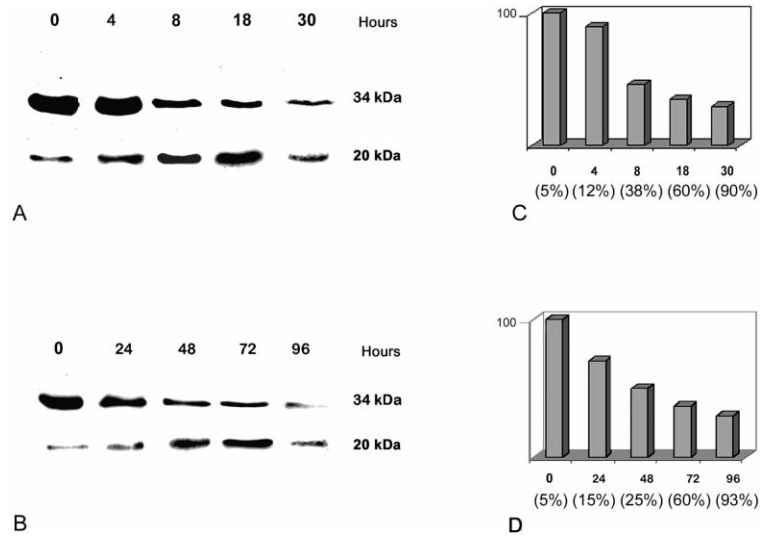

Fig. 1. Time-course of emerin proteolysis in C2C12 myoblasts. C2C12 myoblasts were incubated with staurosporine (A,C) or serum-starved for the specified time-points (B,D). Whole cell extracts were analyzed by SDS–PAGE. 90 μg of proteins were loaded in each lane and immunoblots were revealed using goat polyclonal anti-emerin. The 20 kDa band represents the cleaved emerin fragment. C,D: The densitometric analysis of the 34 kDa immunoblotted bands shown in (A,B), respectively; values are reported in arbitrary units as percentage of controls (time-point 0). The same pattern of proteolysis is maintained, despite the different experimental conditions. The rate of apoptotic cells per each time-point, evaluated by DAPI staining of nuclei, is reported in parentheses.

that C2C12 myotubes treated with staurosporine were necrotic, a control sample performed by treatment with 10% ethanol [7] was loaded in the polyacrylamide gel; the emerin amount was not lowered in this sample (Fig. 2A, lane N). Lamin A/C and PARP cleavage were analyzed in the same samples (Fig. 2B) to show induction of apoptosis. A precise correspondence was observed between emerin proteolysis and lamin A/C cleavage; lamin A/C cleavage was an early event, as low molecular weight peptides (about 37 kDa) appeared even at the early time-points of staurosporine treatment

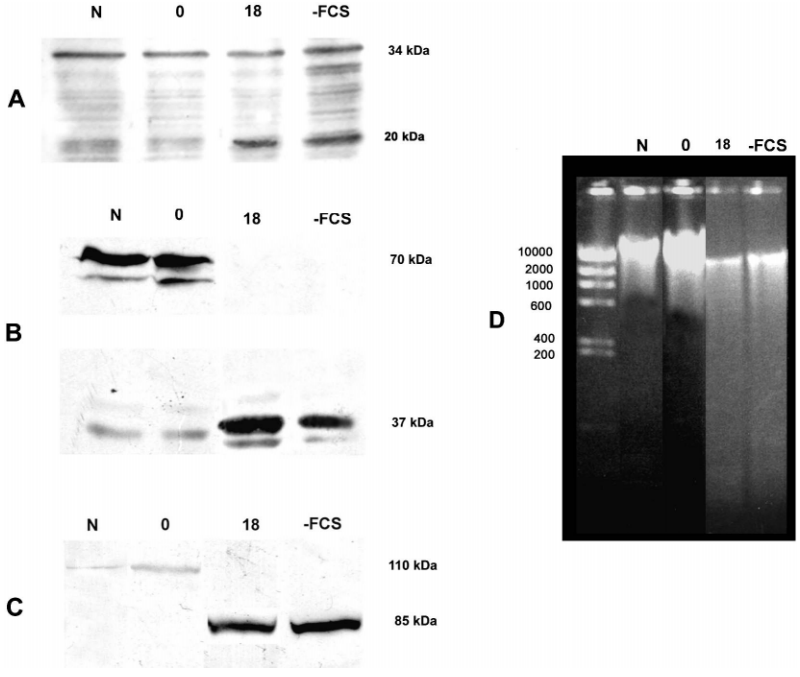

Fig. 2. Emerin proteolysis in C2C12 myotubes treated with staurosporine for 18 h or serum-starved (−FCS) for 72 h. A: Immunoblotted bands were obtained after SDS–PAGE of whole cell extracts and detection by anti-emerin polyclonal antibody. Note that emerin proteolysis does not occur in necrotic ethanol-treated cells (N). B: Lamin A/C cleavage was observed in the same samples, and native lamin A/C bands disappeared in apoptotic samples, while emerin proteolysis was not complete at the same time-points (18 h of staurosporine treatment and 72 h of serum starvation). Anti-lamin A/C polyclonal antibody was employed to reveal immunoblotted bands. C: PARP cleavage was analyzed in the same samples. The 85 kDa active enzyme is detectable in apoptotic myotubes, while the 110 kDa pro-enzyme is faintly labeled by the antibody employed and it is only represented in control untreated and necrotic cells. Molecular weight markers are reported on the right. D: The DNA ladder of apoptotic nuclei is shown. DNA fragmentation is evident at 18 h of staurosporine treatment or 72 h of serum starvation. Molecular size markers are expressed as number of base pairs.

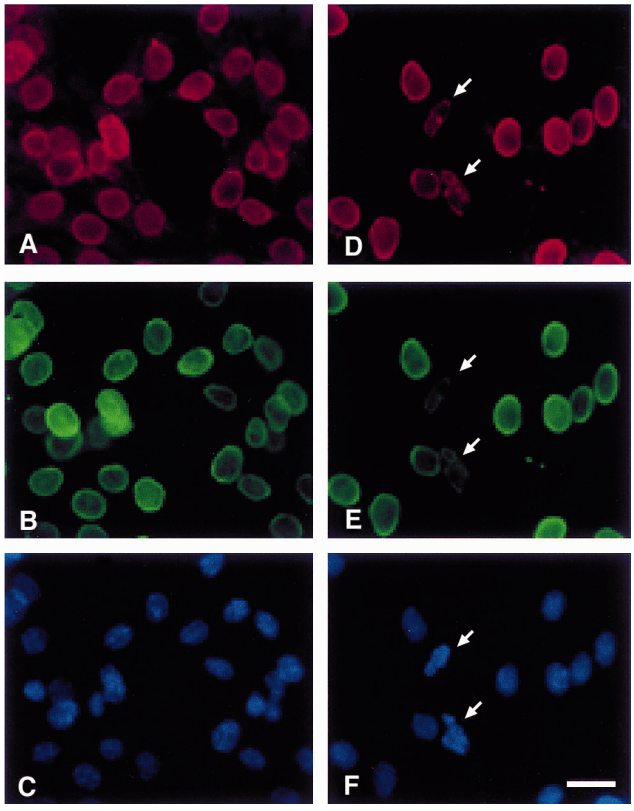

Fig. 3. Emerin-labeling at the nuclear rim is strongly reduced in apoptotic nuclei. Control C2C12 myoblasts are shown in (A–C). Myoblasts treated with staurosporine for 8 h are shown in (D–F). Cells fixed with 2% paraformaldehyde were labeled with anti-emerin monoclonal antibody (A,D) and anti-lamin A/C polyclonal antibody (B,E). DAPI staining (C,F) allows detection of condensed and fragmented apoptotic nuclei indicated by arrows. Bar, 10 μm.

(Fig. 2B). In myotubes treated with staurosporine for 18 h native lamin A/C band almost disappeared (Fig. 2B); this finding was consistent with the strong reduction of the 34 kDa emerin band observed in the same samples (Fig. 2A). Cleaved PARP was detected at any time-point of staurosporine treatment and in serum-starved myotubes, while only the 110 kDa inactive enzyme was observed in control and ethanol-treated cells (Fig. 2C). Fig. 2D shows the DNA ladder analysis of apoptotic myotubes. Oligonucleosomal DNA cleavage was observed after 18 h of staurosporine treatment or after 72 h of serum starvation of cultures.

3.3. Immunocytochemistry

Both staurosporine-treated and serum-starved cells were reacted with anti-emerin monoclonal antibody and anti-lamin A/C polyclonal antibody to evaluate the intensity of labeling and the localization of emerin in apoptotic myoblasts and myotubes. Fig. 3 shows double-labeled myoblasts after 8 h staurosporine treatment. A reduction of emerin-labeling (Fig. 3D) and of lamin A/C staining (Fig. 3E) was clearly evident in the cells with condensed chromatin (Fig. 3F), typical of apoptotic nuclei. The same decrease of emerin staining was observed in myotubes (not shown).

3.4. Electron microscopy

Electron microscopy analysis of apoptotic C2C12 nuclei showed a similar morphology both in myoblasts and myotubes. Fig. 4A shows the morphology of a myoblast nucleus from a differentiating C2C12 culture. In staurosporine-treated cells chromatin was compacted along the nuclear periphery (Fig. 4B), while the nuclear lamina was still preserved. On the other hand, mitochondria were altered and the cytoplasm was condensed, while the plasma membrane was still intact (Fig. 4B). The nuclear membrane appeared intact with nuclear pores more evident in regions where the compacted chromatin was absent. However, condensed chromatin was not closely attached to the nuclear lamina, as shown in Fig. 4C.

3.5. Role of caspases in emerin proteolysis

We evaluated the involvement of caspase 3 and caspase 6 in the proteolysis of emerin observed during the apoptotic process. As expected, caspase 3 inhibitor Ac-DEVD-CHO and caspase 6 inhibitor Ac-VEID-CHO, employed at 100 μM concentration in C2C12 myoblasts, inhibited PARP and lamin A/C cleavage, respectively (Fig. 5). Emerin proteolysis was completely abolished by both competitor substrates (Fig. 5A,B). Polyclonal anti-emerin antibody revealed a cleaved peptide of 20 kDa in lysates obtained from apoptotic cells, which was not labeled by the monoclonal antibody. This peptide was absent in cell lysates where caspase inhibitors had been added (Fig. 5B, lanes 3 and 4).

3.6. Emerin over-expression in staurosporine-treated myoblasts

Over-expression of wild-type emerin in cycling myoblasts did not increase resistance of transfected cells to apoptosis. The percentage of TUNEL positive nuclei within transfected myoblasts was not statistically different from that observed in controls (not shown).

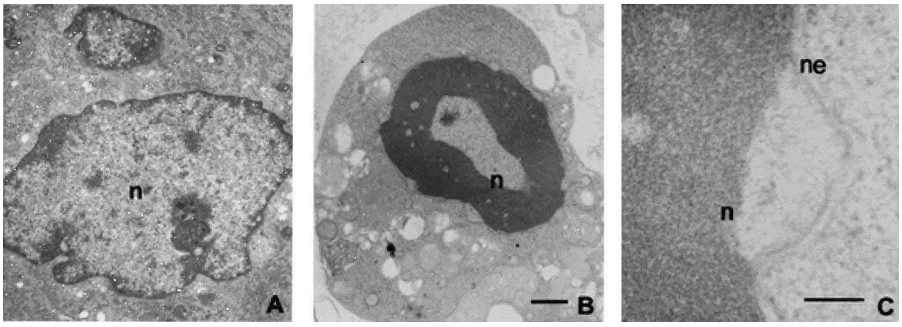

Fig. 4. Electron microscopy analysis of the nuclear envelope in apoptotic C2C12 cells. A control nucleus is shown in (A). Myoblasts treated with staurosporine for 18 h have a high degree of chromatin condensation (B). The detachment of the nuclear envelope from chromatin is shown at higher magnification in (C). n, nucleus; ne, nuclear envelope. Bars, 500 nm (A,B) and 10 nm (C).

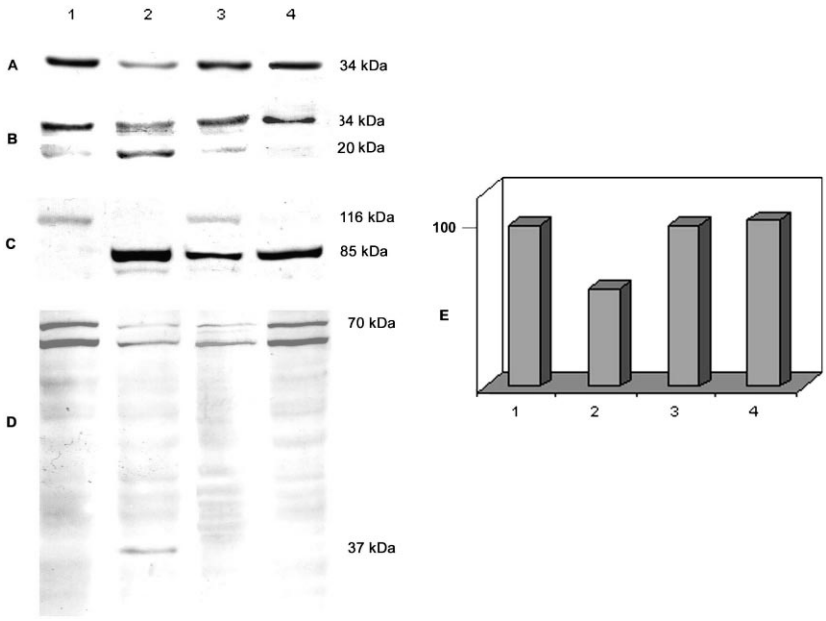

Fig. 5. Effect of caspase inhibitors on the in vivo proteolysis of emerin, PARP, and lamin A/C. A: C2C12 myoblasts were incubated for 2 h in the presence of 100 μM Ac-DEVD-CHO (lane 3) and 100 μM Ac-VEID-CHO (lane 4), then in the presence of staurosporine for an additional 8 h. Control cells are shown in lane 1, apoptotic cells not treated with caspase inhibitors are shown in lane 2. Emerin proteolysis has been revealed in this immunoblot by monoclonal anti-emerin antibody which fails to detect any cleavage peptide. Both caspase inhibitors completely abolished emerin proteolysis. B: Emerin cleavage is detected, in the same samples shown in (A), by a polyclonal anti-emerin antibody raised against the C-terminus of the mouse protein. A cleaved peptide of approximately 20 kDa is revealed. Both caspase 3 and caspase 6 inhibitors strongly reduce the amount of cleaved peptide (lanes 3 and 4). C: PARP proteolysis is analyzed by an immunoblotting performed on the same samples shown in (A,B); note that caspase 3 inhibitor strongly reduces PARP cleavage (lane 3). D: Lamin A/C cleavage is analyzed in the same samples as in (A–C). The 37 kDa product of lamin A/C proteolysis is detected in apoptotic myoblasts (lane 2). Caspase 6 inhibitor Ac-VEID-CHO completely abolishes lamin A/C proteolysis (lane 4). E: The densitometric analysis of immunoblotted bands (34 kDa) labeled with anti-emerin monoclonal antibody and here reported in A was performed. Results are reported as percentage of control (non apoptotic cells) densitometric value (1) and represent the values for apoptotic myoblasts (2), apoptotic myoblasts treated with Ac-DEVD-CHO (3) and apoptotic myoblasts treated with Ac-VEID-CHO (4). The values are representative of three different experiments.

4. Discussion

In the present study we observed that: (1) cleavage of emerin occurs in apoptotic myoblasts and myotubes and gives rise to a low molecular weight peptide of about 20 kDa; (2) emerin-labeling at the nuclear rim decreases upon the onset of chromatin condensation and almost disappears at the late stage of apoptosis; (3) inhibition of caspase 3 and caspase 6 activity completely abolishes emerin proteolysis in apoptotic nuclei suggesting a direct involvement of caspase 6 in emerin processing during apoptosis.

Involvement of nuclear envelope proteins, LAP2β [7], Nup 153 [7], LBR [9] and lamins [6] in the programmed cell death has been demonstrated. The fate of emerin in apoptotic cells was previously investigated in Jurkat lymphoid cells [8]. The protein is not cleaved in this cellular model, suggesting that emerin plays a role in maintaining the association of chromatin with the nuclear envelope during the early stages of apoptosis [8]. LBR, as well as emerin, was not proteolyzed in human lymphoblastoid cells [7], but it was cleaved in apoptotic avian cells [9] and in human HeLa nuclei following mistargeting of lamin B and PP1 [34]. Using two different protocols to induce apoptosis in C2C12 cells, we could detect emerin proteolysis. Comparison of DNA ladder and emerin Western blot analyses shows that complete emerin cleavage occurs when DNA fragmentation is evident. Cleavage of lamin A/C in apoptotic myotubes appeared to follow a more rapid kinetic than emerin processing, as shown in Fig. 2B. In fact, complete proteolysis of native lamin A/C preceded disappearance of the 34 kDa emerin band. This feature of apoptotic nuclei suggests a role for emerin in maintaining nuclear integrity until the late stages of programmed cell death.

The immunoblotting analysis performed with anti-emerin polyclonal antibody which is raised against the C-terminus of murine emerin, shows that emerin cleavage in apoptotic nuclei gives rise to one peptide of low molecular weight. This fragment is not detected by the monoclonal anti-emerin antibody, probably because the amino-terminal end of the protein detected by this antibody is not contained in the cleaved peptide.

In embryonic fibroblasts obtained from mice null for the lamin A/C gene, emerin is displaced from the nuclear rim and it is redistributed in the cytoplasm [20]. This behavior would have suggested a re-localization of emerin even in apoptotic cells, where lamin A/C is cleaved. Our data concerning the fate of emerin during programmed cell death, demonstrate that the amount of emerin is strongly reduced in apoptotic nuclei, but cytoplasmic staining is not increased. This feature suggests that emerin processing in apoptotic nuclei is not only dependent on lamin A/C cleavage.

Therefore, we investigated a possible involvement of caspases [35–38] in emerin cleavage during programmed cell death. The results reported here show that emerin proteolysis is related to both caspase 3 and caspase 6 activity. Active caspase 3 activates caspase 6 with a cascade pattern [38];

thus, inhibition of emerin cleavage by both competitor substrates strongly suggests that caspase 6 is directly involved in emerin cleavage. Nevertheless, caspase 6 is known to cleave lamin A/C. Therefore, inhibition of emerin proteolysis by caspase 6 substrate, suggests either a correlation between lamin A/C cleavage and emerin processing in apoptotic nuclei or a direct interaction of the protease with emerin. The binding site of emerin on the lamin A/C sequence has been identified in the tail domain of lamin A (amino acids 384–566) [39], while the caspase 6 cleavage site corresponds to Asp230 in the lamin A sequence [6]. Thus, the site of interaction between emerin and lamin A is not affected in caspase-cleaved lamin A, and emerin binding should not be impaired. The identification of caspase 6 as the emerin protease is supported by the presence of consensus aspartic acid containing sequences [37,38] for caspase 6 within the nucleoplasmic domain of emerin. Cleavage of emerin at V^{70}DSD would generate a fragment of approximately 26 kDa, while cleavage at V^{126}DAD would generate a fragment of about 19 kDa, which fits with the molecular weight of the observed proteolytic product. An anti-emerin polyclonal antibody [33] raised against the N-terminus of the protein allowed us to detect a fragment of 26 kDa in apoptotic myoblasts, which is absent from lysates obtained after in vivo caspase 3 or caspase 6 blockage (not shown). Thus, we cannot rule out the possibility that emerin is cleaved at V^{70}DSD. However, further studies, including mutations within the possible caspase cleavage motifs, are needed in order to characterize the in vivo cleavage site for caspase 6 in the emerin sequence. It is worth noting that emerin cleavage at one or more of the caspase 6 consensus sequences here reported should affect the emerin domain involved in lamin A/C interaction (amino acids 1–188) [16,21,39]. Thus, emerin proteolysis could facilitate the breakdown of the nuclear lamina–nuclear matrix that occurs during programmed cell death [40,41], provided that emerin is linked to these structures in living cells [18]. Moreover, the detachment of nuclear envelope from chromatin here observed in apoptotic nuclei (Fig. 4) has been related to cleavage of inner nuclear membrane proteins [41], and could also involve emerin proteolysis. It is tempting to speculate that also processing of cytoplasmic structures in apoptotic muscle cells could be facilitated by emerin proteolysis; in fact, we previously reported an extra-nuclear localization of the protein in differentiating myoblasts [27] and hypothesized an involvement of emerin in cytoskeleton organization; moreover, an impaired organization of egg cytoplasm in *Drosophila* due to mutations in a nuclear lamin has been recently described [42].

Over-expression of wild type emerin in myoblasts did not elicit a protective effect against apoptosis induction; the suggested absence of a survival role of emerin in myogenic cells, is consistent with our results showing the same rate of programmed cell death in EDMD cells carrying a null mutation in the emerin gene, as in control human cells (not shown). This finding also agrees with cleavage of emerin in apoptotic muscle nuclei being a late event not involved in triggering of the apoptotic nuclear mechanisms.

The results reported here describe the fate of emerin in apoptotic myoblasts. It should be interesting to evaluate the processing of the protein in other cell types undergoing programmed cell death, to investigate whether the biological mechanism here described is shared by different tissues or it is a feature of myoblasts. In this respect, the absence of emerin processing in lymphoid T cells undergoing programmed cell death [8] should support the latter hypothesis. It is worth noting that an apoptosis repressor selectively expressed in myoblasts has been reported [43] and this could suggest the existence of apoptotic pathways specific for muscle cells.

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