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SDS-dependent cleavage of nuclear DNA into high molecular weight DNA fragments: a signal to the engagement of apoptosis?

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In this paper we employed an extraction of the cells with high concentration of sodium chloride (a procedure commonly used for preparation of histone-depleted nuclei) to investigate the genesis of nuclear DNA degradation during apoptosis. We demonstrated that apoptosis in primary culture of murine thymocytes and in continuously growing Swiss 3T3 fibroblasts is associated with progressive disintegration of nuclear DNA into high molecular weight (HMW) DNA fragments of about 50—150 kb followed by the development of oligonucleosomal DNA ladder. In apoptotic cells both HMW-DNA cleavage and internucleosomal DNA fragmentation can be detected either by cell treatment with ionic detergents (SDS) or by extraction with high concentration of sodium chloride. However, at the early stage of apoptosis only SDS-detected HMW-DNA cleavage can be observed which precedes the sodium-chloride-detected nuclear DNA degradation. SDS-detected but not sodium-chloride-detected formation of HMW-DNA fragments occurs in apoptotic cells as early as before detachment. It may be observed also in nonapoptotic cells after they reach the confluent state and is reversible. On the basis of obtained results it is possible to suggest that SDS-detected HMW-DNA cleavage represents a physiological reaction of alive cells that accompanies an early commitment step of apoptosis.

Introduction. Apoptosis is a genetically regulated form of cell death, in which superfluous or abnormal cells are eliminated from an organism [1]. Apoptosis (also referred to as a programmed cell death) plays a fundamental role in cell homeostasis and in a variety of physiological and pathological processes [2, 3]. Although apoptosis occurs in diverse cell types and is triggered by a number of extracellular and intracellular signals it involves the uniform morphological changes of the cells such as chromatin condensation, cytoplasmic vacuolization and plasma membrane blebbing [2]. The morphological alterations of apoptosis are accompanied by a variety of biochemical changes. Elevations in cytosolic free calcium (rew [4]) and cytosolic hydrogen ions [5] are followed by internucleosomal DNA degradation [6, 7] and sharp decreases in cellular NAD levels [8—10]. Despite considerable effort has been directed toward defining the biochemical basis for morphological changes associated with apoptosis, no consensus has been achieved. Activation of endonuclease which preferentially cleaves DNA at internucleosomal regions has been considered as a critical event in apoptosis for many years [2, 3]. More recently, the attention of many laboratories has been focused on another essential step of apoptosis, namely, activation of proteases [rews 11, 12].

In the early phases of apoptosis peculiar modifications of nuclear morphology become evident. Morphological nuclear changes typical for apoptosis usually coincide with double-strand cleavage of DNA at internucleosomal sites [2, 3]. Recent evidence suggests, however, that the changes in nuclear morphology observed during apoptosis seem to be more closely correlated with the proteolysis of a subset of nuclear proteins [13—18] and the onset of high molecular weight (HMW) DNA fragmentation, an event that has been shown to precede [19—22], or, in some cell types, to occur in the absence of internucleosomal DNA cleavage [23—25]. The formation of HMW-DNA fragments, possibly resulted from the cleavage of looped DNA domains at the attachment points on the nuclear scaffold, and the activation of proteases, which seem to act in parallel

with nucleases [16—18], have been postulated to be an early critical event in apoptosis.

Although considerable progress has been achieved in understanding the biochemical events, underlying morphological changes in apoptotic cells, remarkably few is known about initiating events that trigger apoptotic cell death. The observation that apoptosis-related cytoplasmic changes can be achieved in enucleated cells [26, 27] suggests that nuclear events are not essential for initiation of apoptosis. Genotoxic damage, however, is a powerful apoptotic stimulus, and it seems likely that the nucleus contains important mediators which can initiate apoptotic cascade, at least for particular apoptotic inducers.

In this paper we employed a procedure of cell extraction with high concentration of sodium chloride to monitor the integrity of nuclear DNA during apoptosis and demonstrated that before the formation of HMW-DNA fragments, revealed in sodium-chloride-extracted cells, the «hidden» HMW-DNA cleavage takes place in apoptotic cells, that can be detected by cell treatment with SDS. SDS-dependent HMW-DNA cleavage in apoptotic cells occurs at the early stage of apoptosis, is observable in the density-arrested nonapoptotic cells, and is reversible.

Experimental Procedures. *Cell lines and culture conditions.* Murine thymocytes obtained from the thymus of a 4—5 week old mouse (line BALB/C) and Swiss 3T3 fibroblasts were used for this investigation. 3T3 fibroblasts were routinely incubated in DMEM medium supplemented with 10 % fetal calf serum (FCS) in an atmosphere of 95 % air, 5 % CO_2 to give a final suspension of $5 \cdot 10^6$ cells/ml. Apoptosis in exponentially growing 3T3 fibroblasts was induced by serum withdrawal. Thymocytes primary cultures were prepared from intact thymocytes to give a final suspension of $5 \cdot 10^6$ cells/ml in RPMI 1640/10 % FCS and incubated under conditions indicated above for at least 6 h either with or without 1 mM dexamethasone.

Morphological examination of cells. Cells were harvested by centrifugation and resuspended in 100 μl of phosphate buffered normal saline (PBS) at $\approx 10^7$ cells/ml followed by staining with 1 μg/ml of Hoechst 33342 for 30 min at 37 °C. After washing cells were resuspended in 30 μl of PBS and fixed with 30 μl of 4 % paraformaldehyde. For inspection of chromatin 10 μl aliquots were laid on glass slides coated with 3-aminopropyl-triethoxysilane. Microscopy was performed under conditions of normal illumination and fluorescent light using phase contrast optics.

DNA analysis in cellular preparations. 200 μl of thymocyte suspension containing app. $1 \cdot 10^6$ cells were placed into the well of cell culture plate and equal volume of 1 % low-melting point agarose in PBS-buffer preheated to 37 °C was added. For preparation of DNA samples from 3T3 fibroblasts cultural medium was collected and detached cells were harvested from the cultural medium by centrifugation. Attached cells were scraped in the fresh medium and collected by centrifugation. Both attached and detached cells were resuspended in 200 μl of the fresh medium and embedded into agarose by addition of preheated to 37 °C equal volume of 1.5 % low-melting point agarose prepared on PBS-buffer. Agarose-embedded cells (both thymocytes and fibroblasts) were either treated with the lysing buffer (PBS-buffer containing 10 mM EDTA, 1 % SDS) for 1 h at 25 °C or with the extraction buffer (20 mM tris-HCl, pH 7.5, 1 mM EDTA, 0.2 mM PMSF, 2 M NaCl) three times for 20 min at 4 °C. Both SDS-treated and sodium-chloride-extracted embedded in agarose cells were washed three times for 20 min at 25 °C with washing buffer (extraction buffer without NaCl) and subjected to electrophoresis.

Agarose gel electrophoresis Agarose plugs with SDS-treated- and sodium-chloride-extracted cells were subjected to conventional or field inversion gel (FIGE) electrophoresis. Conventional gel electrophoresis was carried out in 1.2 % agarose at 70 V for 3—4 h using 0.5 × TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8.5). As a molecular weight marker was used 1 kb ladder, obtained from Life Technologies. FIGE was performed in 1 % agarose at 85 V for 18 h in 0.5 × × TBE buffer under constant pulses of electric field (24 s «forward» and 8 s «backward») allowing resolution of DNA molecules sized up to 500 kb [47]. In some cases FIGE was carried out for 5—6 h allowing resolution of both low- and high molecular weight DNA. After electrophoresis, the gel was stained with 1 μg/ml ethidium bromide for 10 min, viewed using a UV transilluminator and photographed using Micrat 300 film.

Results. Data presented in Fig. 1 show that dexamethasone-induced apoptosis in primary culture of thymocytes is associated with an extensive cleavage of nuclear DNA into high molecular weight (HMW) DNA fragments of about 300 kb and 50—150 kb (300 kb-DNA fragments represent an compression artifact under FIGE employed, results not shown). Parallel fractionation of SDS-treated apoptotic cells both by field-inversion- and conventional gel electrophoresis indicates that the formation of HMW-DNA fragments takes place at the early stage of apoptosis (Fig. 1), and precedes the internucleosomal DNA fragmentation, which represents a hallmark of apoptosis in these cells.

As is evidenced from the results presented in Fig. 2 the typical pattern of nuclear DNA disintegration in apoptotic thymocytes can be detected either by treatment of agarose-embedded cells with ionic detergent

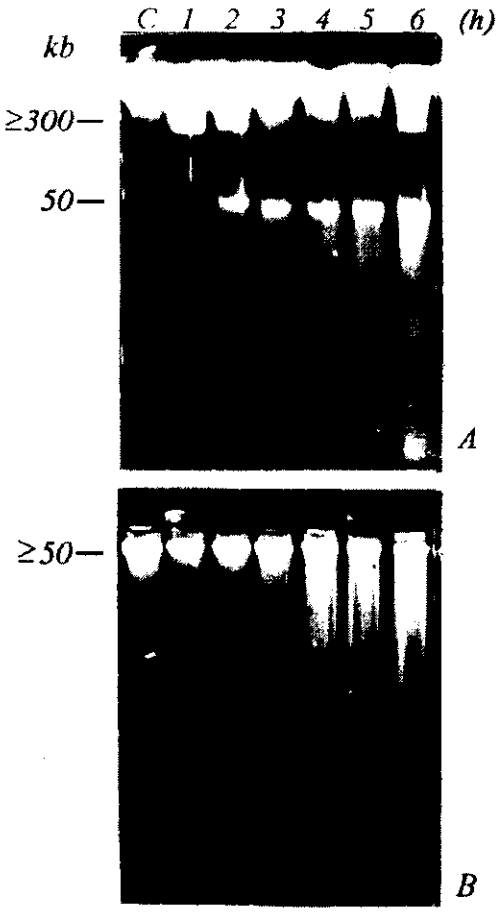

Fig. 1. The pattern of nuclear DNA cleavage in murine thymocytes induced to undergo apoptosis with dexamethasone. Intact murine thymocytes were resuspended in RPMI medium supplemented with 10 % FBS and allowed to incubate for 1—6 h in the presence of 1 μM dexamethasone. At different time points the samples of the cells were embedded in low-melting agarose as described in Experimental Procedure. Agarose-embedded cells were treated with 1 % SDS for 1 h at 37 °C and fractionated either by FIGE (Panel *A*) or by conventional gel electrophoresis (panel *B*). *C*, control cells, non-treated with dexamethasone

SDS or by cell extraction with high concentration of sodium chloride (a procedure commonly used for preparation of histone-depleted nuclei). However, the formation of SDS-detected HMW-DNA fragments in apoptotic thymocytes precedes the nuclear DNA fragmentation detected by high-salt-extraction (Fig. 2, *A*). Incubation of primary thymocyte culture without apoptotic inducers also results in similar pattern of nuclear DNA disintegration, which involves the formation of HMW-DNA fragments and concomitant development of oligonucleosomal DNA ladder (Fig. 2, *B*). This indicates that isolated thymocytes are committed to death, and die via apoptosis even in the absence of apoptotic inducers. At the same time the formation of HMW-DNA fragments to occur rapidly in the cells committed to apoptosis can be detected only in SDS-treated- but not in high-salt-extracted cells, while the oligonucleosomal DNA ladder can be detected by both cell treatment with SDS and extraction with high concentration of sodium chloride (Fig. 2, *B*). The examination of nuclear morphology by Hoechst 33342-staining reveals that internucleosomal DNA fragmentation accompanies the apoptotic changes in nuclear morphology, while the SDS-detected HMW-DNA cleavage is not associated with the apoptosis-related chromatin changes (Fig. 2).

Data presented in Fig. 3 show that SDS-detected HMW-DNA cleavage in apoptotic thymocytes is reversible after brief heat treatment of the cells. The reversibility of HMW-DNA cleavage is observed at the early stage of apoptosis with its subsequent loss at the advanced stage coinciding with the development of oligonucleosomal DNA ladder (Fig. 3).

The absence of HMW-DNA cleavage in SDS-non-treated thymocytes as well as reversibility of HMW-DNA cleavage at the early stage of apoptosis allow us to suggest that SDS-detected HMW-DNA cleavage is not associated with the apoptotic DNA degradation but represents an early genomic event in cells predisposed to undergo apoptosis. To further investigate the relationship between the HMW-DNA cleavage and apoptotic DNA degradation we examined the integrity of nuclear DNA in continuously growing Swiss 3T3 cells induced to undergo apoptosis by serum deprivation. To facilitate the DNA analysis a conventional gel-electrophoresis has been used instead of FIGE since it allows to detect easily both internucleosomal and HMW-DNA fragmentation (latter is detected as a single condensed band sized about 50 kb, Fig. 1).

Data presented in Fig. 4 demonstrate that the progressive accumulation of HMW-DNA fragments and concomitant development of oligonucleosomal ladder, detected either by cell treatment with SDS or extraction with sodium chloride, is restricted to the detached serum-deprived 3T3 cells. In attached cells, however, there is no obvious sodium-chloride-detected DNA fragmentation but SDS-detected HMW-DNA cleavage may be observed. These data indicate that the formation of SDS-detected HMW-DNA fragments in apoptotic 3T3 cells precedes sodium-chloride-detected HMW-DNA cleavage and occurs in alive (attached) cells committed to undergo apoptosis.

From the data presented at Fig. 5 it is seen that the SDS-detected formation of HMW-DNA fragments takes place in attached 3T3 cells in the absence of apoptotic inducers, with the maximal efficiency of fragmentation being in confluent cells. At the same

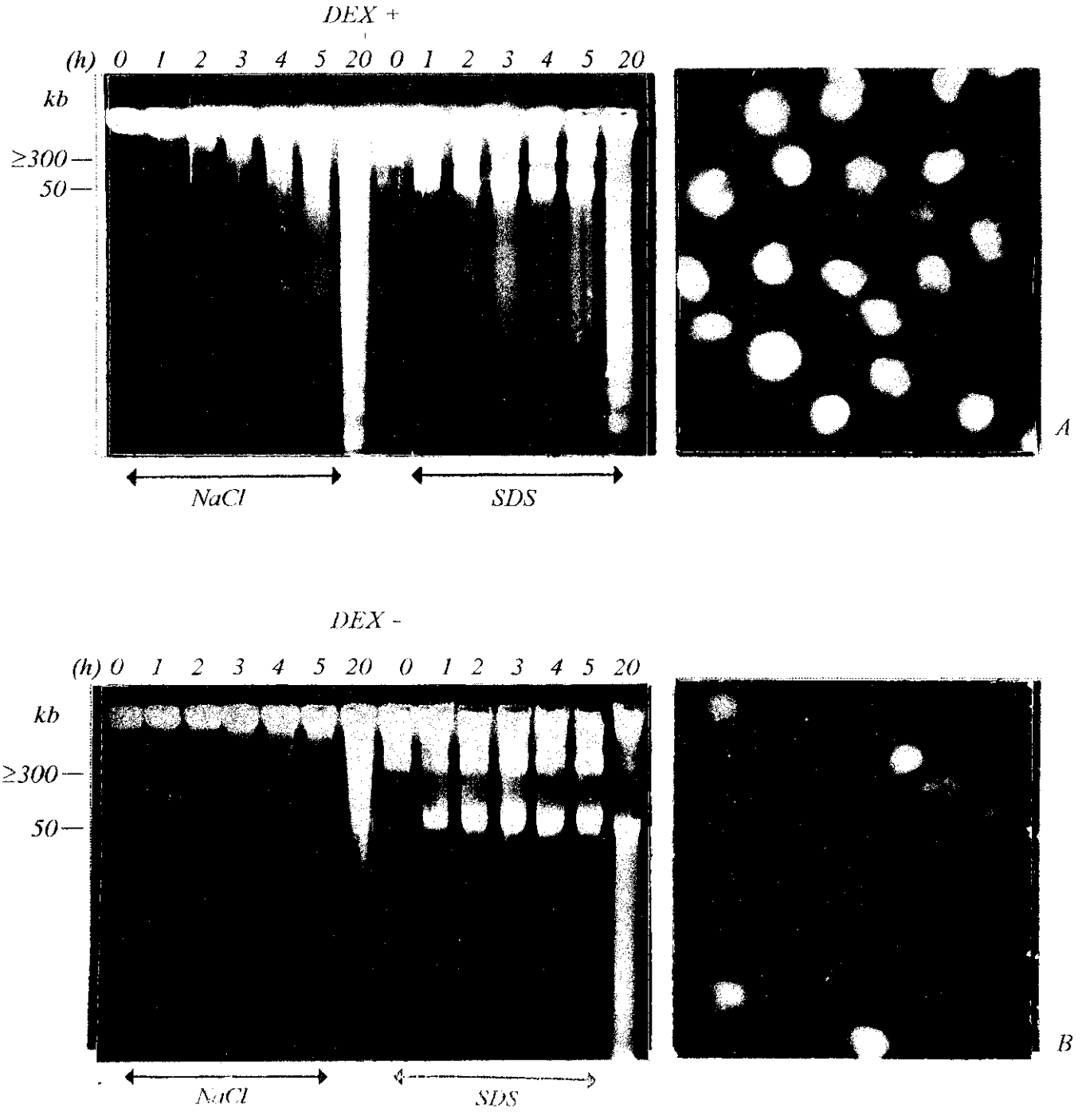

Fig. 2. The pattern of nuclear DNA cleavage in apoptotic thymocytes and thymocytes committed to undergo apoptosis. Intact murine thymocytes were incubated at 37 °C for 0–20 h either with (panel *A*) or without (panel *B*) 1 μM dexamethasone. After incubation the cells were embedded in low-melting agarose as described in Experimental Procedure and then were extracted either with 2 M NaCl or treated with 1 % SDS (as pointed on the figure). Both sodium-chloride-extracted and SDS-treated cells were washed three times with washing buffer (see Experimental Procedure) and fractionated by FIGE. Left panels demonstrate the HMW-DNA fragments separated by FIGE (ethidium bromide staining). Right panels show the morphological features of chromatin in Hoechst 33342-stained thymocytes after 5 h of cell incubation

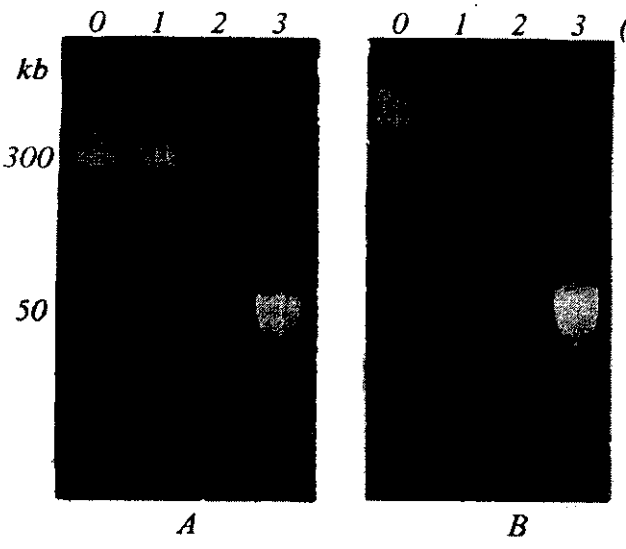

Fig. 3. The reversibility of HMW-DNA cleavage in thymocytes at the early stages of apoptosis. Thymocytes were treated with 1 μM dexamethasone and allowed to incubate for 0—3 h. At different time points cells were embedded into agarose as described in Experimental Procedures (panel *A*) or were additionally incubated at 55 °C for 10 min followed by agarose embedding (panel *B*). After gelation cells were treated with SDS and fractionated by FIGE as described in Experimental Procedures

time in detached cells, which appear after monolayer has been established in attached cells, the HMW-DNA cleavage can be revealed by both cell treatment with SDS and extraction with sodium chloride (Fig. 5, *B*).

Data presented suggest that SDS-detected formation of HMW-DNA fragments represents a physiological reaction of alive cells that accompanies changes in functional activity of the cells during the cell passage.

Discussion. Our results show that apoptosis in primary culture of murine thymocytes and in continuously growing 3T3 fibroblasts is accompanied by an ordered fragmentation of nuclear DNA. Two stages of nuclear DNA disintegration can be distinguished in apoptotic cells: the formation of HMW-DNA fragments followed by the development of oligonucleosomal DNA ladder (Fig. 1, 4). At the same time our data indicate that there are two types of HMW-DNA cleavage in cells induced to undergo apoptosis, namely, the HMW-DNA cleavage detected either with NaCl or SDS and that detected only by cell treatment with SDS.

Because most of the histones and non-histone proteins are extracted upon treatment of cells with high concentration of sodium chloride [28—34] one can expect that ds DNA breaks should be revealed in high-salt-extracted cells. As an illustration both HMW-DNA fragments and oligonucleosomal DNA ladder can be detected in high-salt-extracted cells at the advanced stage of apoptosis (Fig. 2, 4) and is

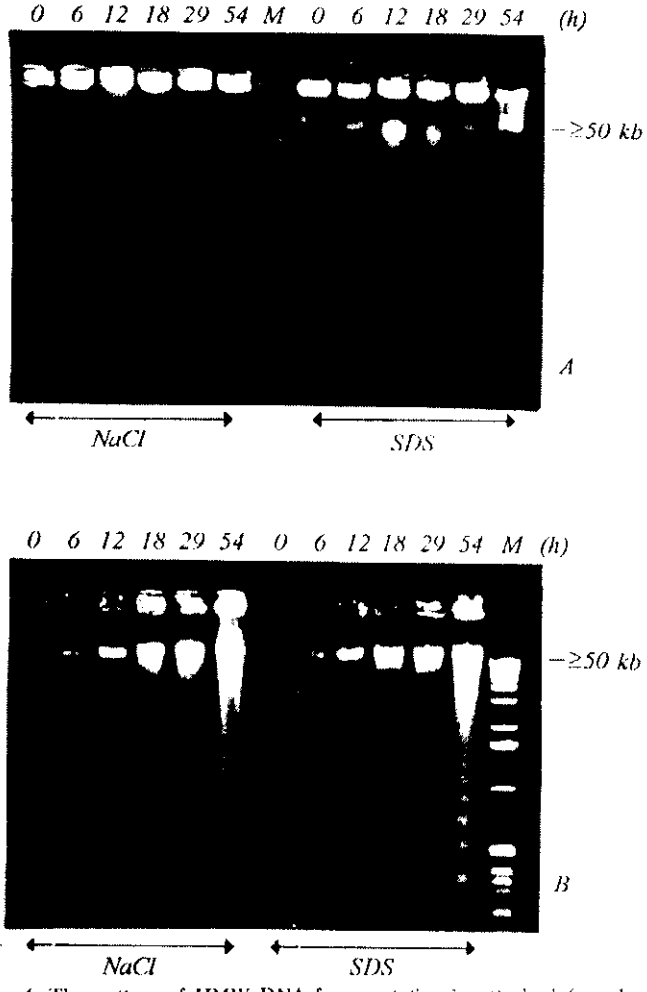

Fig. 4. The pattern of HMW-DNA fragmentation in attached (panel *A*) and detached (panel *B*) 3T3 fibroblasts induced to undergo apoptosis by serum deprivation. Exponentially growing cells (50 % of monolayer) were incubated in serum-deficient medium for 0—54 h. Following incubation the cultural medium was collected and detached cells were harvested from the cultural medium by centrifugation. Attached cells were scraped in the fresh serum-deficient medium and collected by centrifugation. Both attached and detached cells were embedded into agarose as described in Experimental Procedures followed by extraction either with 2 M NaCl or 1 % SDS. Both sodium-chloride- and SDS-extracted cells were washed three times with washing buffer (see Experimental Procedure) followed by fractionation by conventional gel electrophoresis. M, molecular weight standard, 1 kb-DNA ladder

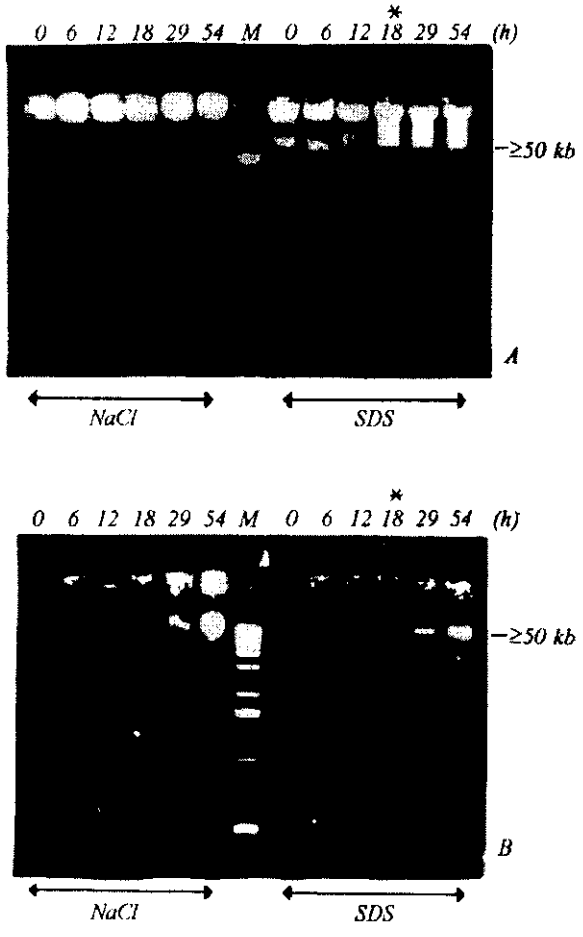

Fig. 5. The pattern of HMW-DNA fragmentation in 3T3 fibroblasts in the absence of apoptotic inducers. The conditions of experiment and the designations are the same as described in the legend to Fig. 4 with the only difference that cells were incubated in serum-containing medium. Asterisk indicates the time point when monolayer has been established

further confirmed by the results of Lichtenstein et al. [57] that treatment of isolated nuclei with DNAse I releases the nuclear DNA upon extraction of nuclei with 2 M NaCl. The absence of the HMW-DNA cleavage in high-salt-extracted thymocytes, cultured *in vitro* without apoptotic inducers, as well as in thymocytes at the early stage of dexamethasone-induced apoptosis (Fig. 2) clearly indicate that there are no preexisting ds DNA breaks in the cells committed to undergo apoptosis or at the early stage of apoptosis. At the same time the detection of HMW-DNA cleavage in the same cells by cell treatment with SDS suggests that the HMW-DNA cleavage is either induced or revealed by addition of detergent. SDS-dependent formation of HMW-DNA fragments makes the phenomenon observed strikingly analogous to the topoisomerase II-dependent DNA cleavage. This is further confirmed by the observation that SDS-detected HMW-DNA cleavage at the early stage of dexamethasone-induced apoptosis in murine thymocytes is reversible under conditions that favor topoisomerase II-dependent rejoining reaction [58] (Fig. 3). It is of interest that the reversibility of SDS-detected HMW-DNA cleavage in dexamethasone-treated thymocytes is lost at the advanced stage of apoptosis, when oligonucleosomal DNA ladder is developed (Fig. 3). This suggests that different nucleases may be involved in the HMW-DNA cleavage at the different stages of apoptosis. Alternative explanation is that apoptosis-related proteolysis of topoisomerase II [24, 48] could underlie the loss of reversibility of HMW-DNA cleavage at the advanced stage of apoptosis.

The examination of nuclear DNA disintegration in 3T3 fibroblasts, induced to undergo apoptosis by serum deprivation, allows us to divide several stages of apoptotic DNA fragmentation as follows. SDS-detected formation of HMW-DNA fragments proceeds in attached cells at the early stage of apoptosis, then the detachment of the cells takes place, which is associated with the sodium-chloride-detected HMW-DNA cleavage, followed by the development of oligonucleosomal DNA ladder. In the consecutive events of apoptotic DNA degradation to occur in 3T3 cells the appearance of sodium-chloride-detected HMW-DNA fragments seems to be attributed to the detached (dead) cells (Fig. 4). At the same time the SDS-detected HMW-DNA cleavage, which precedes the sodium-chloride-detected formation of HMW-DNA fragments in apoptotic thymocytes (Fig. 2), occurs in attached serum-deprived 3T3 cells (Fig. 4) as well as in the confluent serum-non-deprived cells (Fig. 5). Furthermore, the SDS-detected HMW-DNA cleavage is reversible at the early stages of apoptosis upon brief heat treatment of the cells (Fig. 3) and after addition of the serum to the serum-deprived cells [49]. All these data suggest that SDS-detected formation of HMW-DNA fragments shares no common feature with an apoptotic DNA degradation but represents an early genomic event in cells committed to undergo apoptosis. Indeed, there is no difference in the ability to SDS-detected formation of HMW-DNA fragments both in attached serum-deprived cells (preapoptotic cells) and serum-non-deprived (healthy) cells (compare Fig. 4 and 5). Remarkably, an increased formation of SDS-detected HMW-DNA fragments takes place in cells stimulated to undergo apoptosis by non-genotoxic inducers, namely by dexamethasone, and by serum deprivation. This indicates that the nucleus contains important mediators of the early events of apoptosis even in the absence of DNA damaging agents.

It is becoming increasingly evident that apoptosis

occurs in response to many different stimuli, of which a partial list includes withdrawal of growth factors, inappropriate expression of genes that stimulate cell cycle progression, and viral infection. Several of these factors have in common the property that they alter the cell cycle, and it has been proposed that apoptosis primarily reflects a cell cycle perturbation [50, 51]. Because the most probable event accompanying a cell engagement to apoptosis might be an arrest of cell proliferation one could assume that SDS-detected formation of HMW-DNA fragments reflects changes in proliferative status of the cells. This is further confirmed by the data that monolayer establishment in continuously growing 3T3 cells is accompanied by an increased formation of SDS-detected HMW-DNA fragments (Fig. 6). On the other hand, the stimulation of confluent cells to proliferation by dilution of monolayer is accompanied by decreased HMW-DNA cleavage [49]. These data allow us to suggest that an arrest of cell proliferation may underlie the formation of SDS-detected HMW-DNA fragments, an event that seems to represent an early commitment step to the subsequent apoptosis.

The cleavage of nuclear DNA into HMW-DNA fragments to occur at the early stage of apoptosis (or at the commitment stage of apoptosis) clearly indicate that there is a periodic location of cleavage sites along genomic DNA. It is becoming apparantly evident that in the interphase nucleus each chromosome occupies its own three-dimensional space due to the established contacts with the protein scaffold to generate a complex structural organization. An extraction of nuclei with high concentration of sodium chloride reveals that nuclear DNA is organized into topologically constrained loop domains anchored to a protein backbone structure referred to as the nuclear matrix or chromosome scaffold [28—34].

Topoisomerase II has been shown to be a major component of the nuclear matrix and plays an important role in chromosome structure and dynamics [35—37]. Several lines of evidence suggest that topoisomerase II is concentrated in a number of discrete anchoring complexes which probably form the bases of the chromatin loop domains [35, 38—40, rew 41, 42].

Previously we demonstrated that the HMW-DNA fragments, resulted from the treatment of nuclear DNA preparations with the protein-denaturing agents, correspond to DNA loop domains [52]. The relation of HMW-DNA fragments to the DNA loop domains were also concluded by other authors [53, 54]. We presented evidence indicating that DNA loop domains in nonapoptosizing tissues are involved in functional topoisomerase II/DNA complexes with the ability to mediate the cleavage/rejoining reactions [55, 56]. Based on studies with purified topo II enzymes and DNA, a two-stage model for topoisomerase II-mediated cleavage/religation reactions has been proposed [45, 46]. According to this model, an enzyme/DNA cleavable complex is the key covalent intermediate in the topoisomerase II-mediated DNA turnover which is in rapid equilibrium with the noncleavable complex [45, 46]. DNA in the cleavable complex is broken, but ds DNA breaks are hidden since they are clamped by topo II enzyme. The exposure of the cleavable complex to protein denaturants such as SDS or alkali results in a cleaved DNA product involving the covalent linking of topoisomerase II subunits to the 5'-ends of broken DNA [45, 46].

Proceeding from the results that structural domains of nuclear DNA contribute to the functional topoisomerase II/DNA complex [55, 56] it seems appropriate to interpret the SDS-detected HMW-DNA cleavage to occur at the early stage of apoptosis as a transition of DNA structural domains from «noncleavable» to «cleavable» state, mediated by topoisomerase II. Our results allow us to suggest that changes in the functional properties of the complexes topoisomerase II/DNA structural domains, resulting in altered pattern of HMW-DNA cleavage, may be a physiologically valuable genome reaction, that underlies an early commitment step of apoptotic cell death.

To summarize it can be concluded that changes in the integrity of nuclear DNA, recognizable as an altered pattern of SDS-dependent HMW-DNA cleavage, represent a physiological reaction of the cells engaged in apoptosis. This reaction may be associated with changes in proliferative status of the cells and may be interpreted as a transition of topoisomerase II/DNA complex between «cleavable» and «noncleavable» states.

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В. Т. Солов'ян

DS-Na-залежне розщеплення ядерної ДНК на високомолекулярні фрагменти: сигнал до початку апоптозу?

Резюме

У роботі застосовано метод високосолевої екстракції інтактних клітин для вивчення динаміки розщеплення ядерної ДНК при апоптозі. Показано, що апоптоз в первинній культурі тимоцитів і культивованих прикріплених клітинах фібробластів миші супроводжується прогресивною деградацією ядерної ДНК на високомолекулярні фрагменти розміром 50—150 тис. п. н. з подальшим формуванням олігонуклеосомних фрагментів. В апоптозних клітинах розщеплення ядерної

ДНК на високомолекулярні та олігонуклеосомні фрагменти відбувається як при обробці клітин DS-Na, так і при високосолевій екстракції. Однак на ранніх етапах апоптозу має місце лише DS-Na-залежна високомолекулярна фрагментація ядерної ДНК, яка передує формуванню високомолекулярних фрагментів, що виявляються при високосолевій екстракції клітин. На відміну від розривів, які виявляються високосолевою екстракцією, DS-Na-залежне розщеплення ядерної ДНК на високомолекулярні фрагменти спостерігається в апоптозних клітинах перед вспливанням, а також має місце в неапоптозних клітинах, що досягли моношару. Показано, що DS-Na-залежне розщеплення ядерної ДНК на високомолекулярні фрагменти на початкових стадіях апоптозу є зворотним в умовах, що сприяють залежному від топоізомерази II лігуванню розривів ДНК. Отримані результати свідчать, що DS-Na-залежне розщеплення ядерної ДНК на високомолекулярні фрагменти є фізіологічною реакцією живих клітин, яка передує апоптозній деградації ядерної ДНК.

В. Т. Соловьян

DS-Na зависимое расщепление ядерной ДНК на высокомолекулярные фрагменты: сигнал к началу апоптоза?

Резюме

В работе применен метод высокосолевой экстракции интактных клеток для изучения динамики расщепления ядерной ДНК при апоптозе. Показано, что апоптоз в первичной культуре тимоцитов и культивированных прикрепляющихся клетках фибробластов мыши сопровождается прогрессивной деградацией ядерной ДНК на высокомолекулярные фрагменты размером 50—150 тыс. п. н. с последующим формированием олигонуклеосомных фрагментов. В апоптозных клетках расщепление ядерной ДНК на высокомолекулярные и олигонуклеосомные фрагменты происходит как при обработке клеток DS-Na, так и при высокосолевой экстракции. Однако на ранних этапах апоптоза имеет место лишь DS-Na-зависимая высокомолекулярная фрагментация ядерной ДНК, предшествующая формированию высокомолекулярных фрагментов, выявляемых высокосолевой экстракцией клеток. В отличие от разрывов, обнаруживаемых высокосолевой экстракцией, DS-Na-зависимое расщепление ядерной ДНК на высокомолекулярные фрагменты наблюдается в апоптозных клетках перед всплытием, а также имеет место в неапоптозных клетках, достигших монослоя. Показано, что DS-Na-зависимое расщепление ядерной ДНК на высокомолекулярные фрагменты на начальных стадиях апоптоза является обратимым в условиях, способствующих опосредованному топоизомеразой II лигированию разрывов ДНК. Полученные результаты свидетельствуют, что DS-Na-зависимое расщепление ядерной ДНК на высокомолекулярные фрагменты представляет собой физиологическую реакцию живых клеток, которая предшествует апоптозной деградации ядерной ДНК.

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