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Spontaneous Premature Chromosome Condensation, Micronucleus Formation, and Non-Apoptotic Cell Death in Heated HeLa S3 Cells

Ultrastructural Observations

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Hyperthermia is an efficient means of inducing cell death in vivo and in vitro. Among human neoplastic cells, HeLa S3 cells are susceptible to heat injury when exposed to long duration moderate hyperthermia (41.5 C), conditions that are reproducible and sustainable in the clinical setting. Hence, HeLa S3 cells are a useful substrate for evaluation of hyperthermic injury in human neoplasia. Previous studies have demonstrated a consistent response of HeLa S3 cells to moderate hyperthermia: spontaneous premature condensation of chromosomes during heat exposure in S phase followed by apparent nuclear fragmentation and, inevitably, cell death. To further characterize the morphological features of this process, HeLa S3 cells grown in suspension at 37 C were heated for 4, 8, 12, or 16 hours at 41.5 C and harvested in glutaraldehyde for electron microscopic evaluation. Compared with untreated controls, heated samples exhibited a characteristic pattern of chromosome condensation that mimicked mitotic prophase but was followed by haphazard asymmetric segregation of chromatid clusters in abnormal metaphase/anaphase and premature reformation of nuclear membrane, resulting not in nuclear fragmentation, but in multiple micronuclei. This pattern of nuclear morphology was not observed in controls. The fraction of cells with micronuclear morphology increased with time in heated samples (from 3.6% at 4 hours to 16.6% at 16 hours), consistent with previous light microscopic analyses of nuclear fragmentation. Cells with multiple micronuclei subsequently exhibited features similar to necrotic cell death. Apoptosis was never observed. Moderate hyperthermia appears to induce a novel morphological pattern of cell injury and death in HeLa S3 cell lines that may be useful as a means of screening cell lines for nonmorphological analyses of hyperthermic injury. (Am J Pathol 1995, 146:963–971)

Hyperthermia has been used as means of inducing cell injury and death in a variety of normal and neoplastic immortalized cell lines derived from both human and animal tissues. Observations on the basis of these findings have served both as an impetus toward and a validation of clinical trials of hyperthermia as a mode of cytotoxic therapy in human neoplasia.[1,2]

Certain cell lines exhibit thermotolerance (resistance to further cell killing during prolonged exposure to heat) under conditions of moderate, long duration hyperthermia (exposure to temperatures ranging from 41.5 to 42.5 C,[3–7] temperatures that are similar to those used in initial clinical trials for human malignancy[1,2] and that (unlike temperatures of 43 C or higher) can be reproducibly achieved *in vivo* in human tissues.[8,9] However, analyses of HeLa S3 cells

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suggest that thermotolerance does not develop in response to this level of thermal exposure.[10,11] Investigations of other human cell lines have demonstrated the commonality of the HeLa cell response.[12,13] Hence, these cells may be a useful model for evaluating the effects of chronic hyperthermia in human neoplasms.

Morphological evaluation for cells that become thermotolerant at 41.5 C, such as Chinese hamster ovary (CHO) cells[3,5,6] and those that do not become thermointolerant at this temperature, including HeLa S3 cells,[11,13] have been performed at the light microscopic level, suggesting that a common pattern of cell injury and cell death may occur during thermal treatment. After exposure to 41.5 C during S phase, a proportion of cells undergo spontaneous premature chromosome condensation followed by what morphologically resembles abnormal mitosis and nuclear division without cytokinesis, resulting in a pattern of nuclear changes that has been described as nuclear fragmentation. This phenomenon is distinct from both necrotic and apoptotic cell death and differs from the so-called premature chromosome condensation, or prophasing, that occurs when the nuclear membrane of an interphase cell breaks down after fusion with a cell in metaphase.[6,14–16] To further study this pattern of cell injury in heat-sensitive cells, we analyzed the light microscopic and ultrastructural aspects of heat-related cell alterations in HeLa S3 cells. The resultant data suggest that a consistent pattern of morphological alteration occurs in this model, one that may be applicable to the study of other human neoplastic cell lines.

Materials and Methods

Cell Cultures

HeLa S3 cells were grown at 37 C in suspension culture on Joklik-modified minimal essential medium containing 10% iron-supplemented calf serum and antibiotics (500 U/ml sodium penicillin G and 500 μg/ml streptomycin sulfate). Cells were maintained in suspension culture at 1×10^5 to 3×10^5 cells/ml.

Heat Treatment

Cells were heated in suspension in a Thelco incubator (Precision Scientific Co, Chicago, IL) that was modified in the author's laboratory (MAM). Before the start of the experiment, spinner flasks containing HeLa S3 cells prepared as described were immersed in 41.5 C water baths to decrease the warm-up transient. Flasks were then transferred to the incubator and maintained at 41.5 C. Temperature was regulated with a YSI proportional controller (Yellow Springs Instrument Co., Yellow Springs, OH), and air inside the incubator was circulated by use of a blower fan, which maintained air temperature within ±0.05 C during the course of the experiment. Culture flasks were removed from the incubator at 4-hour intervals up to a total of 16 hours of heat exposure. A flask of cells maintained at 37 C for the duration of the experiment served as a normal control.

Light and Electron Microscopy

After removal of flasks from the incubator at 4, 8, 12, and 16 hours, cell samples were immediately washed in phosphate-buffered saline by centrifugation and resuspension and were again spun down to a soft pellet (400 × *g* for 5 minutes at 4 C). Phosphate-buffered saline was decanted off the cell pellet, the cells were resuspended in 3% buffered glutaraldehyde in sodium cacodylate buffer (0.1 mol/L, pH 7.4) and centrifuged under the above stated conditions in a conical tube. The cells remained in glutaraldehyde at room temperature for not more than 24 hours. The fixed cell pellets were divided into 1 mm^3 fragments, rinsed in cacodylate buffer, postfixed in osmium tetroxide (2% w/v) and stained *en bloc* with uranyl acetate. The pellet fragments were partially dehydrated in 70 and 95% ethanol and infiltrated with a hydrophobic resin (Spurr, Polysciences, Inc., Warrington, PA). The resin was polymerized by exposure to heat (70 C for 7 hours).

One-micron sections prepared from each sample were stained with toluidine blue. Cell morphology and mitotic phase were scored in at least 275 cells (constituting all cells in a single section from a representative block from each sample). Cells were scored as interphase when a single intact nucleus was present; binucleate and multinucleate cells (with interphase-type chromatin dispersal and each nucleus measuring more than 5 μ) were scored separately. Mitotic prophase required chromosome condensation and a visible nuclear membrane. Metaphase required near linear alignment of chromatids on one or more equatorial planes and no visible nuclear membrane. Anaphase was scored when chromatids were condensed, were segregated into two or more groups, and were not arranged in linear arrays. Bent chromatids, suggestive of a motive force, were not a consistent feature in nuclei scored as anaphase in heat-treated samples. All anaphase nuclei lacking this feature were scored as abnormal. Telophase required complete segregation of chromatid clusters, early

dispersion of chromatin material, and evidence of nuclear membrane reformation with or without evidence of cytokinesis. Cells were scored as having multiple micronuclei (a pattern synonymous with nuclear fragmentation in previously reported analyses; see Refs. 6 and 10) when telophase-like chromatin dispersal and complete reconstitution of nuclear membranes without cytokinesis were evident in four or more small (2 to 5 μ) nuclei.

Thin sections for transmission electron microscopy were mounted on 200-mesh copper or nickel grids, stained with lead citrate, and examined with a Philips CM10 electron microscope.

Results

Light Microscopy

Cells were well preserved at all time intervals studied. General observations on nuclear morphology and mitotic phase are summarized in Table 1.

Untreated controls consisted largely of singly nucleated cells in interphase (87.7%). Relatively few binucleate and multinucleate cells were identified. All mitotic phases were represented in dividing cells, providing a basis for comparison with treated samples. No cells with multiple micronuclei (nuclear fragmentation) were seen.

Over the time course of this study, the number of mononucleate cells progressively decreased after 4 hours, whereas the number of cells in metaphase/anaphase increased. The latter was accompanied by the appearance of cells with multiple micronuclei at 4 hours (constituting 3.6% of cells), with a dramatic increase in micronuclear forms after 12 hours (to a maximum of 16.6% at 16 hours). Binucleate forms were only modestly increased during the study period, whereas cells with multiple interphase nuclei consistently increased after 4 hours.

All cells scored as anaphase in treated samples appeared abnormal, with either asymmetric bipolar or multipolar chromatid clusters. Transitional forms between anaphase nuclei and multiple micronuclei (nuclear fragmentation) were evident at time points after 4 hours. The pattern of chromatin dispersal in the latter often resembled that of normal telophase nuclei. The accumulation of cells in abnormal anaphase at latter time points was in conspicuous contrast to the near absence of cells in morphologically normal telophase.

Beginning at 4 hours, dark staining pigment granules accumulated in the center of mitotic cells. These became more numerous in both mitotic and micronucleated cells with increasing time.

Less than 1% of cells in controls exhibited features of necrotic cell death (nuclear pyknosis, hydropic change, and membrane dissolution). In heated samples, the number of necrotic cells was increased but never exceeded 5% of the sample.

Electron Microscopy

Untreated cells exhibited few if any alterations in ultrastructural morphology. Mitotic phases were normal. Prophase consisted of a progressive condensation of chromatids within an intact nuclear membrane. Metaphase was heralded by dissolution of the nuclear membrane and linear alignment of chromatids along one or more planes (Figure 1A). Anaphase was marked by segregation of chromatids and symmetric division. Telophase was characterized by dispersal of chromatin and reconstitution of nuclear envelope from smooth endoplasmic reticulum (Figure 1B). Because a single plane of section was analyzed for each mitotic event, characteristics of the astral centrioles and microtubular arrays of the mitotic spindle could not be studied in detail. This was also true of heat-treated samples. In all mitotic cells between prophase and anaphase, membrane-bound organelles were excluded from the central cell zone that was occupied by mitotic chromatids; most organelles were uniformly distributed in the periphery of

Table 1. *Cell Morphology and Mitotic Phase*

Hours of hyperthermia	Number of cells (%)									
				Mitotic phase						
	Total	Multiple micronuclei	Interphase	Pro	Meta	Ana	Telo	Total	Binucleate	Multinucleate
Control	400	0 (0)	350 (87.7)	10 (2.5)	7 (1.8)	4 (1.0)	14 (3.5)	35 (8.8)	12 (3.0)	2 (0.5)
4	276	10 (3.6)	242 (87.7)	1 (0.4)	0 (0)	3† (1.1)	2 (0.7)	6 (2.2)	13 (4.7)	5 (1.8)
8	279	21 (7.5)	219 (78.4)	8 (2.9)	2 (0.7)	4† (1.4)	1 (0.4)	15 (5.4)	17 (6.0)	16 (5.7)
12	390	30 (7.6)	271 (69.5)	8 (2.0)	3 (0.8)	30† (7.8)	1 (0.2)	42 (10.8)	30 (7.8)	17 (4.3)
16	307	51 (16.6)	187 (60.9)	4 (1.3)	5† (1.7)	20† (6.5)	0 (0)	29 (9.5)	24 (7.8)	16 (5.2)

Pro, prophase; Meta, metaphase; Ana, anaphase; Telo, telophase.
*Total number (and percentage) of cells in a recognizable (normal or abnormal) mitotic or mitotic-like nuclear phase.
†Cells in metaphase or anaphase in these samples are abnormal in appearance. Note that multiple micronuclei appear to represent the telophase analogue of cells proceeding through abnormal meta/anaphase without subsequent cytokinesis (see text).

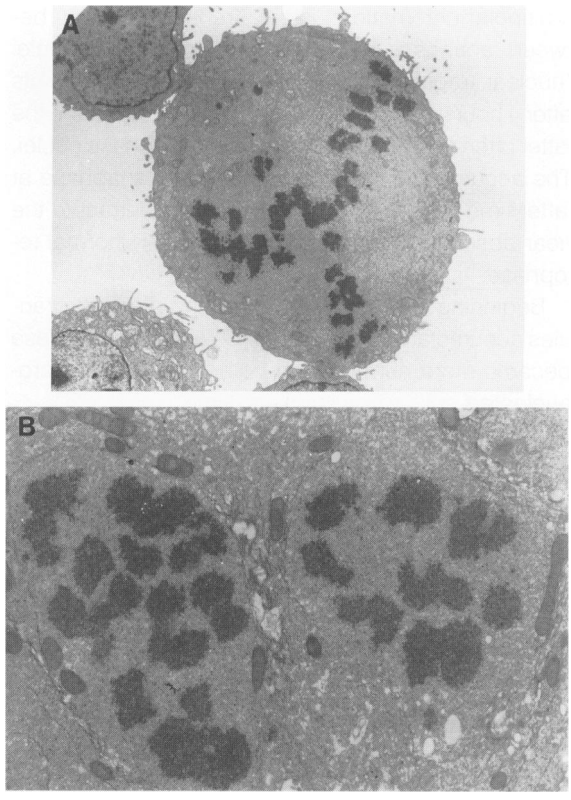

Figure 1. *Mitosis in untreated HeLa S3 cells. Condensation of chromatids in prophase and metaphase is associated with exclusion of organelles from the cell center* (**A**). *During early telophase, mitotically segregated chromatids are invested with a new nuclear envelope* (**B**). *Original magnification:* **A**, *×1900;* **B**, *×7000.*

the cell. Only during late anaphase/telophase did these organelles appear in the center of the cell; most remained peripheral throughout nuclear division.

In heat-treated samples, several abnormalities were observed. The most important related to aberrations in chromatid segregation observed by light microscopy. The earliest nuclear event was most common in 4-hour samples and was characterized by uneven chromatin condensation around nucleoli (Figure 2A). This patten of condensation was never observed in control samples and could not be distinguished from normal early prophase in light microscopic samples. Cells in late prophase appeared similar to those of untreated controls, but by the time the nuclear membrane had dispersed at early metaphase, chromatids were only rarely well organized in a linear array. The distinction between disordered metaphase and early anaphase was thus not always obvious (see Figure 2B).

In both metaphase and phase-indeterminant cells, irregular profiles of bilaminar membrane with fine structural resemblance to smooth endoplasmic reticulum (SER) were loosely admixed with chromatids. In some mitotic-like cells, linear fragments of SER clearly divided chromatids into three or more groups (Figure 2C, D). Comparison with later anaphase nuclei (Figure 3A) suggested that these linear elements of SER provided the substrate for partial or complete reconstitution of nuclear membranes well in advance of the characteristic chromatin changes of telophase. Reconstitution of the nuclear membrane was never observed in metaphase or early anaphase nuclei.

No normal telophase nuclei were observed at any time sample in treated cells. Rather, early division of chromatids by SER in anaphase led to the formation of multiple small nuclei (nuclear fragmentation), each undergoing dispersion of chromatin material in a manner otherwise characteristic of telophase (Figure 3B, C). Cells with multiple micronuclei were progressively more numerous at later time points in this analysis, whereas metaphase and early anaphase nuclei were progressively more difficult to identify. These changes corresponded to light microscopic observations. Notably, in 12- and 16-hour samples, the nuclei in occasional micronucleated cells became contracted, with increased electron density of the nuclear matrix and irregular condensation of chromatin (Figure 3D). These changes were accompanied by generalized organellar damage (dissolution of membranes and hydropic change) and contraction of cytoplasmic volume. This secondary pattern resembled necrotic cell death and mimicked changes seen in a small fraction of interphase cells in all samples studied. Evidence of apoptotic cell death was absent in controls and all heated samples. Although not rigorously evaluated, microtubular elements of the mitotic spindle could not be identified in any treated cell undergoing mitotic-like changes.

For heated samples, in most post-prophase cells at all time points studied, membrane-bound organelles were haphazardly distributed throughout the cell, often becoming admixed with metaphase and anaphase chromatids (Figure 2B). However, organelles were never incorporated into developing micronuclei.

Beginning in the 4-hour time sample, scattered organelles, particularly mitochondria, appeared damaged, with focal disruption of cristae and peripheral membranes. The changes were more common in organelles in a central or perinuclear location. At later time points, similar changes were accompanied first by intralysosomal accumulation of laminar and electron-dense material, then by a preponderance of amorphous electron-dense material, and, finally, accumulation of similar debris in extraorganellar cytoplasm. This material remained localized in a perinuclear distribution or was admixed with condensed chromatids and corresponds to pigment seen by light microscopy and resembled lipofuscin.

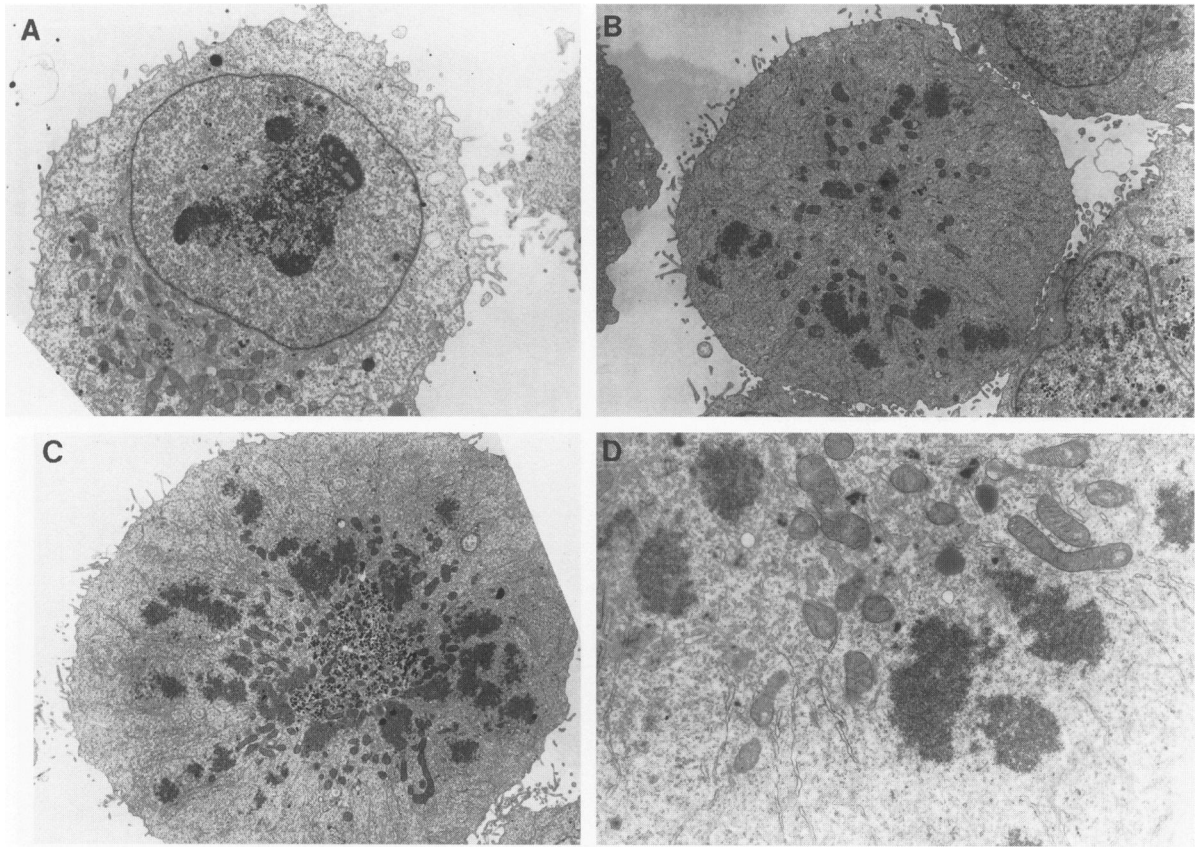

Figure 2. *Chromosome condensation in heat-treated HeLa S3 cells. Early chromatin condensation is uneven and appears to be centered on nucleoli* (A). *In late prophase and metaphase, organelles congregating at the cell center are admixed with chromatids* (B). *In late metaphase and anaphase, linear strands of endoplasmic reticulum appear to segregate the chromatids into several groups* (C *and* D). *Original magnification:* A, ×3810; B, ×2750; C, ×2750; D, ×9800.

Discussion

Studies of heat-induced cell injury and death in human neoplasms and cell lines established from them are impeded by the lack of a model with which to compare observations. Although biochemical data point to characteristic patterns of cell injury during and after exposure to heat,[3–7,10,11] differences between cell types in cycling time, duration of G1, S, and G2 phases, native differentiation-specific cell functions, and tolerance to heat-induced injury may contaminate observations on the mechanism of cell death when various cell lines are compared. In contrast, the sequences of morphological alterations in cells are more generalized with respect to cell proliferation and injury. Reproducible morphological changes between cell lines subjected to similar types of injury may thus provide empirical evidence of a common mechanism of injury.

Morphological studies on heat-induced injury have largely been performed on animal models, with particular emphasis on CHO cells.[3,6] In the case of S phase CHO cells, the light microscopic pattern of cell injury and death is similar to that observed in the present analysis of HeLa S3 cells. In both, the appearance of prophase nuclei in the first nuclear division during heat treatment is similar to that of untreated controls, with condensation of chromatids in a loose nuclear matrix without dissolution of the nuclear membrane. In contrast, metaphase and anaphase nuclei appear poorly organized, with haphazard and often asymmetrical orientation of condensed chromosomes. In S phase CHO cells, abnormal nuclear and cell division ensues in a proportion of cells, although some nuclei appear fragmented after abortive attempts at nuclear division.[6] In HeLa S3 cells analyzed in this study, the latter feature predominates, with few if any cells progressing through mitosis to cell division.[10,11] Light microscopic patterns in timed samples in the present analysis in fact suggest that virtually all cells entering mitosis during the period of heat stress undergo micronuclear formation (nuclear fragmentation), since the ratio of cells in prophase/metaphase to cells with nuclear fragmentation progressively decreases as the exposure time

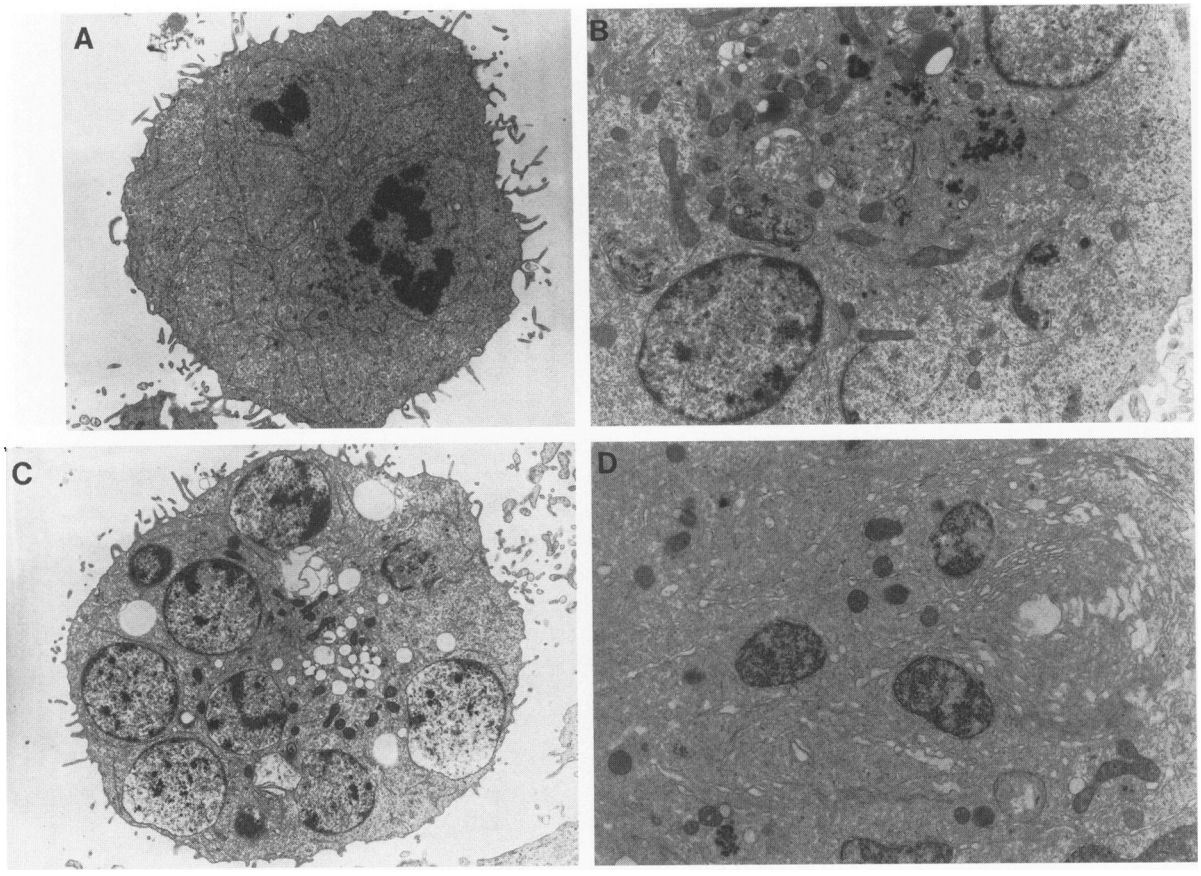

Figure 3. *Micronuclear formation in heat-treated HeLa S3 cells. In later anaphase before dispersion of chromatin, separately packaged chromatids are enveloped by a nuclear envelope* (A). *There is no evidence of a normal telophase in these cells; rather, telophase-like chromatin changes accompany partial* (B) *or complete* (C) *reconstitution of nuclear membrane, resulting in cells with multiple micronuclei (nuclear fragmentation). In 12- and 16-hour samples, micronuclei in some cells undergo coarse chromatin condensation and nuclear contraction similar to pyknosis* (D). *Original magnification:* A, ×3810; B, ×7000; C, ×3810; D, ×7000.

to heat is successively prolonged. The small but persistent increase in cells with two or more normal appearing interphase nuclei suggested that some cells passing through abnormal anaphase were able to reconstitute functional nuclei or had undergone unequal cytokinesis after abnormal chromosome segregation. Lack of symmetry in late telophase would render cells approaching or completing cytokinesis in this state difficult to identify. The differences between cell lines in progression through mitosis are likely due to differences in the way these disparate cell lines respond to chronic low level heat stress. Asynchronous cultures of CHO cells, for example, characteristically exhibit features of thermotolerance (resistance to further cell killing) after prolonged exposure to temperatures similar to those used in the present study (41.5 C),[3,4,6,7] a phenomenon that has been ascribed to blockage of heated cells in G1 phase (synchronous cultures of CHO cells exposed to heat during S phase exhibit little or no thermotolerance.[5,6] In contrast, HeLa S3 cells remain susceptible to heat-associated injury and death during prolonged exposure to this temperature.[10,11] However, comparisons between S phase CHO and HeLa S3 cells also indicate that micronuclear formation increases dramatically in both cell types after heating to 41.5 C for 8 or more hours,[6,10,11] providing evidence that despite thermotolerance in the former cells, the morphological sequence of nuclear change in lethally injured cells is similar.

Differences in ultrastructural changes in CHO cells and HeLa S3 cells, on the basis of a comparison of the present study with an investigation of CHO cells by Coss et al,[3] may also reflect cell-specific features of heat-induced lethal injury, although the latter analyses are problematic. Coss and colleagues[3] described the transmission electron microscopic features of asynchronous CHO cells exposed to 41.5 C for 7 hours; hence, their observation of abnormalities in cells progressing through mitosis and cytokinesis (without conspicuous micronuclear formation) were not fully representative of the spectrum of injury over prolonged thermal injury. Irrespective of this potential shortcoming, they described a feature of thermal in-

jury in CHO cells that is not fully reproduced in the present analysis, ie, a prolongation of metaphase with precocious condensation of nuclear membrane around metaphase chromatids. Notably, the presence of nuclear envelope material did not prevent chromatid segregation or subsequent cell division in these CHO cells.[3] As detailed in this report and elsewhere, precocious condensation of nuclear membrane occurs in heated HeLa S3 cells, but only during the anaphase equivalent, once chromatid clusters have begun to segregate. As expected, the nuclear envelope is reconstituted from membrane elements of the SER; however, in contrast to mitotic cells in untreated controls and normal eukaryotic cells, wherein nuclear membrane reforms around symmetric chromatid clusters in early telophase, the metaphase/early anaphase chromatids are unevenly divided by linear elements of SER and, during abortive attempts at anaphase, they are partially or completely enveloped, forming multiple small nuclei. This process has previously been interpreted at the light microscopic level as evidence of nuclear fragmentation. The pattern of chromatin relaxation and dispersion in each resultant small nucleus resembles that of normal late anaphase and telophase; indeed, in many respects the formation of micronuclei appears to represent the telophase analogue of nuclear division in thermally injured cells. Few if any of the HeLa S3 cells at any time point analyzed appear to have undergone morphologically normal mitosis and cytokinesis. Hence, in contrast to CHO cells, precocious condensation of nuclear membrane as a morphological feature of thermal injury is associated with inhibition of cell division in HeLa S3 cells.

An additional observation of interest in this study is the secondary condensation of chromatin in micronuclei with contraction in nuclear size in 12- and 16-hour samples. These changes are associated with contraction and increased electron density of the cytoplasm by light microscopy and by evidence of organellar membrane injury in electron microscopic samples and appear to be the morphological correlates of cell death. As previous analyses of HeLa S3 cells have indicated, this pattern of injury is associated with cell death after thermal injury.[10]

The metaphase nucleus in heat-treated HeLa S3 cells differs from untreated controls in two other important aspects: 1), early mitotic events are not associated with exclusion of organelles from the cell center, so that mitochondria and other membrane-bound structures are admixed with metaphase and anaphase chromatids and 2), membrane damage to mitochondria and other organelles, either before or during prophase, results first in perinuclear intralysosomal accumulation of lamellar membrane material and then extraorganellar condensation of electron-dense products of phospholipid degradation. This latter phenomenon appears to be identical with the formation of lipofuscin pigment in other cell types. Coss et al[3] described swelling and membrane evaginations in heat-treated metaphase CHO cells but did not observe the formation of electron-dense pigment as a result of these changes; however, the limited time of heat exposure (7 hours) may not have been sufficient for these changes to develop in CHO cells.

Although these changes do not identify the nature or timing of the cell injury, they do seem to be related to specific cellular events that are associated with heat exposure. The premature division of metaphase/anaphase chromatids by nuclear membrane may be related to a dissociation of the control of nuclear division from DNA synthesis. This latter phenomenon has been well characterized in cells exposed to agents that impair DNA synthesis, including hydroxyurea and thermal injury,[10,17] but the mechanism that couples thermal injury to abnormal mitosis has not been fully elucidated. The abnormal segregation of organelles after dissolution of the nuclear envelope in early prophase in HeLa S3 cells is also not clearly related mechanistically to thermal injury, although this haphazard arrangement may support the contention that spontaneous premature chromosome condensation during or after heat exposure occurs in a cell not prepared for cell division. Notably, the earliest pattern of chromatin condensation in treated samples appears abnormal, suggesting that the initiation of this event was also abnormal. Furthermore, given that none of the mitotic events that follow complete chromosome condensation (prophase) in heated HeLa S3 cells appear morphologically normal, it is plausible that the process that leads to micronuclear formation is not a mitotic event at all; rather, it may reflect a mechanistic program of chromatid segregation and nuclear membrane reconstitution that is precipitated by chromosome condensation but is independent of the initiating event (signaling of premitotic events in normal cells). This possibility has been raised elsewhere, on the basis of morphological and biochemical observations.[10] Such an explanation would reasonably account for the lack of symmetric nuclear division and the failure of the cell to proceed through cell division. Although rigorous morphological studies are required for confirmation, the apparent absence of microtubular elements of the mitotic spindle in treated samples may provide corroborative evidence for this interpretation.

The morphological alterations discussed herein differ from the nuclear changes that can be induced

in interphase cells of certain human neoplastic cells (including HeLa cells) by fusion with cells in metaphase. Described as premature chromosome condensation by Johnson and Rao,[13] this latter phenomenon appears to be precipitated by fusion-induced dissolution of the interphase nuclear membrane[13–15] with subsequent aggregation of interphase chromatin/chromosomes in a manner that resembles mitotic prophase. The latter features have prompted the alternative appellation prophasing by Sandberg and colleagues.[14,15] In contrast to hyperthermia-associated spontaneous premature chromosome condensation, which appears to require exposure to heat during S phase, prophasing is an inducible phenomenon that is independent of phase. Nevertheless, the fate of prophased G1 or S phase nuclei is provocatively similar to those of heated HeLa S3 cells. Whereas prophased G2 nuclei contain paired chromosomes that are, in large part, incorporated into the metaphase nucleus as mitosis proceeds, G1-derived chromosomes or S-derived chromatin fragments are often excluded from the normally dividing metaphase nucleus, instead forming micronuclei or chromatin fragments in the cytoplasm of the resulting interphase cell. However, the progressive condensation of chromosomes through an abnormal prophase to a metaphase analogue in heated HeLa S3 cells, the failure of the dividing nucleus to exclude organelles from the aggregated chromosomes in metaphase/anaphase, and the uneven separation of chromosomes by premature reconstitution of nuclear membrane emphasize that the process initiated by heating cells during S phase is nonetheless distinct from prophasing.[5,6,13,14,18]

It is also important to emphasize that the changes observed in HeLa S3 cells do not correspond to other well characterized modes of cell death, ie, necrosis and apoptosis. Generalized plasmalemma and organellar membrane breaks and accumulation of amorphous matrical granules or phospholipid inclusions in mitochondria constitute irreversible changes of ischemic injury and represent the early stages of necrotic cell death. Subsequent interphase nuclear chromatin condensation (pyknosis) heralds the onset of organellar and cytoplasmic hydropic changes, and nuclear dissolution ensues without fragmentation.[19–21] This mode of cell death is mimicked by heated HeLa S3 cells after micronuclear formation but does not account for the primary nuclear abnormality in HeLa S3 cells. Nucleolar dissolution with condensation and layering of chromatin along an intact nuclear envelope in interphase nuclei, features that are characteristic of early apoptosis,[19–21] were not encountered in any cell in the present analysis, and nuclear fragmentation of interphase nuclei was not identified. Notably, apoptotic bodies were not present at any time in heated HeLa S3 cells. The latter observations are of particular importance, as previous analyses of several normal and neoplastic animal and human cell lines suggest that apoptosis may be induced by hyperthermia, particularly in cells that normally exhibit this pattern of cell death (including fetal neuroepithelium in guinea pigs, murine mastocytoma cells, and both human lymphoblastoid and Burkitt's lymphoma cell lines[22–27]), whereas human melanoma cell lines and the carcinoma cell line JAM[24] are apparently not susceptible to programmed cell death during heating. Apoptosis may be induced in CHO cells[22] and a small fraction of HeLa S3 cells,[24] but, in contrast to the present analysis, each such observation has been made in cells exposed to temperatures of at least 43 C. The absence of apoptotic changes in both CHO cells and HeLa S3 cells after prolonged moderate hyperthermia (41.5 C) as in this study, suggests that spontaneous premature chromosome condensation in S phase with micronuclear formation represents a unique, temperature-dependant mode of cell death.

On the basis of the results of this study, we propose that the ultrastructurally defined events that lead to micronuclear formation is a final common pathway that characterizes the response of certain proliferating cells to long duration moderate hyperthermia (41.5 C) and may serve as a morphological model of heat injury in other human cell lines. That this level of hyperthermia can be achieved and sustained in the clinical setting provides additional impetus toward elucidation of the nature of lethal cell injury that occurs under these conditions.[1,2,8] The use of light and transmission electron microscopy may be a sensitive and specific means of screening cell lines for additional biochemical analyses of this clinically important mechanism of cell death.

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