Preapoptotic Chromatin Condensation Upstream of the Mitochondrial Checkpoint*

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When added for a short period (2–4 h) to cells, the kinase inhibitor staurosporine (STS), can trigger double strand breaks, the formation of nuclear foci containing phosphorylated H2AX, Chk2, and p53, a decrease in transcription, and a minor degree of peripheral chromatin condensation. This “preapoptotic chromatin condensation” (PACC) occurs before mitochondrial membrane permeabilization (MMP) and caspase activation becomes detectable and is not inhibited by Z-VAD-fmk or Bcl-2. PACC is followed by classical apoptosis, when cells are cultured overnight, even when STS is removed from the system. After overnight incubation, STS-pretreated cells manifest mitochondrial cytochrome c release, caspase activation, phosphatidylserine exposure, and apoptotic DNA fragmentation. Caspase or MMP inhibitors did not influence the advent of PACC yet did suppress the evolution of PACC toward apoptosis. Importantly, two unrelated MMP inhibitors (viral mitochondrial inhibitor of apoptosis (vMIA) from cytomegalovirus and mitochondrion-targeted Bcl-2) had a larger range of effects than the pan-caspase inhibitor Z-VAD-fmk. Caspase inhibition simply prevented the transition from PACC to apoptosis yet did not reverse PACC and did not restore transcription. In contrast, Bcl-2 and vMIA allowed for the repair of the DNA lesions, correlating with the reestablishment of active transcription. PACC could also be induced by a gross perturbation of RNA synthesis or primary DNA damage. Again, inhibition of MMP (but not that of caspases) reversed PACC induced by these stimuli. In synthesis, our data reveal the unexpected capacity (in particular, Bid), which causes MMP to trigger double strand breaks.

Chromatin condensation and chromatinolysis constitute the classical hallmarks of apoptosis, and both have been thought for long to be both pathognomonic and responsible for apoptotic death (1, 2). Today, it appears clear that full-blown nuclear condensation (pyknosis), formation of nuclear apoptotic bodies (karyorrhexis), and advanced DNA fragmentation (into oligonucleosomes leading to the “apoptotic DNA ladder”) actually are postmortem manifestations, occurring well after the point-of-no-return has been trespassed and irreversible mitochondrial membrane permeabilization and/or explosive caspase activation have sealed the fate of the cell (3–8). Thus, anucleate cells (cytoplasts) can be triggered to manifest cytoplasmic features of advanced apoptosis (such as phosphatidylserine (PS)1 exposure on the plasma membrane and loss of the mitochondrial transmembrane potential (ΔΨm)) in response to, for instance, the general tyrosine kinase inhibitor staurosporine (STS) (9–11), which is a universal, widely used apoptosis inducer (12).

At the biochemical level, apoptosis of mammalian cells is characterized by mitochondrial membrane permeabilization (MMP) and/or massive caspase activation (3–8), which alone or together constitute the point-of-no-return of the lethal signal transduction cascade. Two major pathways can lead to apoptosis (13). The intrinsic (or stress) pathway is triggered by a wide range of inducers including STS, inhibitors of mRNA synthesis (such as actinomycin D), or DNA damage. This pathway critically relies on MMP, which causes bioenergetic failure and the release of potentially cytotoxic proteins from the mitochondrial intermembrane space. Such apoptogenic factors include caspase activators (prototype: cytochrome c, which activates the apoptosis caspase activation complex, once in the cytosol) and caspase-independent death effectors (prototype: apoptosis-inducing factor (AIF), which translocates to the nucleus) (14). MMP is regulated, at least in part, by proteins of the Bcl-2 family (15). The extrinsic (or death receptor) pathway involves activation of plasma membrane receptor of the death receptor family (15). The extrinsic (or death receptor) pathway involves activation of plasma membrane receptor of the death receptor superfamily (e.g. CD95/Apo-1/Fas), leading to the receptor-proximal recruitment of a caspase activation complex (13). The resulting activation of caspase-8 either is sufficient to trigger the proteolytic activation of other caspases or requires the proteolytic activation of proapoptotic proteins of the Bcl-2 family (in particular, Bid), which causes MMP and thus triggers the “mitochondrial amplification loop.”

The characterization of factors responsible for chromatin

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¹ The abbreviations used are: PS, phosphatidylserine; AIF, apoptosis-inducing factor; ΔΨm, mitochondrial transmembrane potential; DiOC6(3), 3,3-dihexyloxacarbocyanine iodide; MMP, mitochondrial membrane permeabilization; NA, nuclear apoptosis; PACC, preapoptotic chromatin condensation; STS, staurosporine; vMIA, viral mitochondrial inhibitor of apoptosis; Z, N-benzoxycarbonyl; fmk, fluoromethylketone; CMV, cytomegalovirus; STAT, signal transducers and activators of transcription; ATM, ataxia telangiectasia-mutated; siRNA, small interfering RNA; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling.
condensation and chromatinolysis has yielded important insights into the pathophysiology of cellular demise. Caspase-dependent DNase (16) as well as series of caspase-independent effectors such as DNase II (17), endonuclease G (18), AIF (19), cyclophilin A (20, 21), acinus (22), and phospholipase A2 (23) have been reported to cause chromatin condensation and nuclear DNA fragmentation, to a variable degree. Thus, caspase-dependent DNase, DNase II, and endonuclease G cause oligonucleosomal DNA fragmentation (16–18), whereas AIF and cyclophilins cause large scale DNA fragmentation to ~50 kbp (19, 20). Similarly, caspase-dependent chromatin condensation is far more pronounced than that induced by AIF, which is more peripheral (19, 24). Accordingly, some authors have distinguished “true apoptosis” (caspase-dependent), with full-blown chromatin condensation, from “apoptosis-like cell death” (caspase-independent) with partial chromatin condensation (25, 26). Similarly, there have been attempts to distinguish an early “stage I” chromatin condensation (AIF-dependent) from a later “stage II” (caspase-dependent) (27).

When carefully examining the morphology of HeLa cells treated with a short pulse (2–4 h) of STS, we found a rather partial “preapoptotic chromatin condensation” (PACC), which occurred before the mitochondrial checkpoint had been activated and before caspase activation through the intrinsic pathway was detectable. Biochemical and functional analysis revealed that STS-induced PACC involved double strand breaks, which occurred before apoptosis and independently from apoptosis because it was not affected by a range of MMP and caspase inhibitors. Importantly, however, MMP inhibitors (but not caspase inhibitors) allowed cells with PACC to repair their DNA and to return to a normal nuclear morphology. These findings reveal unexpected effects of STS on genome integrity. Moreover, MMP inhibition appears to be more effective in triggering DNA repair than caspase inhibition.

MATERIALS AND METHODS

Cell Lines and Culture Conditions—HeLa cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, 10 mM Hepes, and 100 units/ml penicillin/streptomycin at 37 °C under 5% CO₂. Furthermore, HeLa cells were stably transfected with pcDNA3.1 control vector (neomycin), with human...
FIG. 2. PACC occurs upstream of the MMP checkpoint. A, HeLa cells treated with STS for 3 h alone (3h, STS) or with Z-VAD-fmk (3h, STS + zVAD) or STS pulse-treated HeLa cells recultured for 18 h in the absence (3h, STS + 18h) or presence of Z-VAD-fmk (3h, STS + 18h, zVAD) were directly stained with DiOC₆(3) and hydroethidine (HE) or fixed and permeabilized followed by staining with antibodies recognizing activated Bak (Bak a), cytochrome c (Cyt c), or the p17/p19 fragment of proteolytically activated caspase-3 (Casp-3a) and counterstained with Hoechst 33342. B, quantitative analysis of the data obtained as in A. The phenotypic characteristics (X ± S.D., n = 4) of cells with PACC or NA, generated as indicated, were measured as in A. p values were calculated to assess the effect of Z-VAD-fmk as compared with controls cultured in the absence of this inhibitor. *, p < 0.05; #, p < 0.005.

FIG. 3. Analysis of DNA degradation and double strand breaks. After the indicated regimes of STS/Z-VAD-fmk (zVAD) treatment (as described in the previous figure legends), nuclear DNA was subjected to pulse field gel electrophoresis (A) or, alternatively, to ethanol fixation and 4',6-diamidino-2-phenylindole staining for the cytofluorometric determination of subdiploidy (X ± S.D., n = 3) (B). In addition, the cells were subjected to a comet assay (C), and the frequency of comet assay-positive cells (D) was determined by microscopic observation (X ± S.D., n = 3). p values were calculated to assess the effect of Z-VAD-fmk as compared with controls cultured in the absence of this inhibitor. *, p < 0.05.

FIG. 4. Analysis of transcription. Cells were transiently transfected with the luciferase gene (under the control of the CMV promoter in A or under the control of a series of transcription factors in B) were exposed to the indicated combinations of STS ± Z-VAD-fmk (zVAD, 3 h only in B), and the expression of luciferase was measured. This experiment was repeated three times, and means ± S.D. are shown. Significance was calculated with the paired Student’s t test with respect to untreated controls. *, p < 0.05; #, p < 0.005. CREB, cAMP-response element-binding protein.
Bcl-2 (Bcl-2), or with the cytomegalovirus UL37 exon 1 gene (vMIA (viral mitochondrial inhibitor of apoptosis), provided by Dr. V. Goldmacher, ImmunoGen, Inc, Cambridge, MA) (28). Derivatives of the HCT116 cell line (parental, p53−/− or p21−/−) (29) were a gift by D. Vogelstein and cultured in RPMI medium supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, and 100 units/ml penicillin/streptomycin at 37 °C. Cells were cultured in RPMI medium supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, and 100 units/ml penicillin/streptomycin at 37 °C.

**Electron Microscopy**—Cells were fixed for 1 h at 4 °C in 2.5% glutaraldehyde in phosphate buffer (pH 7.4), washed, and fixed again in 2% osmium tetroxide before embedding in Epon. Electron microscopy was performed with a transmission electron microscope (model EM902; Carl Zeiss MicroImaging, Inc.), at 80 kV, on ultrathin sections (80 nm) stained with uranyl acetate and lead citrate.

**DNA Fragmentation**—For pulse field gel electrophoresis, DNA was prepared from agrose plugs (2 × 10^6 cells) followed by electrophoresis in a Bio-Rad CHEF-DR II (1% agarose, Tris-borate-EDTA, 200 V, 24 h, pulse wave 60 s, 120° angle). Double-stranded DNA breaks were assessed by comet assays performed using a kit from Trevigen. Briefly, cells were immobilized in a bed of low melting point agarose, followed by a gentle cell lysis; cleaved DNA fragments migrated out of the cell under electrophoresis.

**Transcription Assay**—Transcription was monitored by an overnight transient transfection of control plasmids (pTA-LUC and pTAL-LUC) or plasmids containing luciferase reporter gene coupled to CMV promoter (pCMV-LUC) or specific cis-DNA sequence (cAMP-response element-binding protein, NFκB, AP1, STAT, and p53, Mercy Pathway Profiling Systems, Clontech). Protein extracts in equivalent protein concentration samples were mixed with 100 μl of substrates (luciferase reporter assay kit, Clontech). Luciferase activity was determined at 25 °C after 10 s with a luminometer (Lumat LB9507, Berthold Technologies GmbH & Co., Bad Wildbad, Germany) and expressed as fold induction of transcription as the ratio of luciferase activity units as parameter on FACS Vantage (BD Biosciences).

**RESULTS AND DISCUSSION**

**PACC in STS-treated Cells**—HeLa cells exposed to a short pulse (3 h) of the general apoptosis inducer STS manifested ruffling of nuclear membranes, accompanied by a partial peripheral chromatin condensation. This phenomenon, which we termed PACC, was well detectable by electron microscopy (Fig. 1A) as well as by staining with the chromatin-specific dye Hoechst and paraformaldehyde-fixed cells, which were stained with 4′,6-diamidino-2-phenylindole (2.5 μg/ml; Molecular Probes) for 30 min at 37 °C. Cellular shrinkage determination was performed by analysis of forward side scatter parameter on FACS Vantage (BD Biosciences).

**Immunofluorescence and Flow Cytometry**—HeLa and HCT116 cells were cultured on coverslips coated with poly-l-lysine and nuclei or mitochondria were stained by 2 μM Hoechst 33342 (Sigma) or 3,3′,5′-triethylbenzimidazolylcarbocyanine iodide (2 μM; Molecular Probes), respectively, before fluorescence microscopic assessment (32). TUNEL staining was performed with a detection kit from Roche Applied Science GmbH & Co., Bad Wildbad, Germany) and expressed as fold induction of transcription as the ratio of luciferase activity units as parameter on FACS Vantage (BD Biosciences).

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Hoechst 33342 (Fig. 1B). It was detectable well before major shrinkage was detectable, before PS residues were exposed on the plasma membrane surface (detectable with annexin V-FITC) and before cells lost their viability (and thus became permeable to the vital dye propidium iodide). However, if cells that had been pulse-treated with STS (3 h) were washed and cultured overnight (18 h), they manifested hallmarks of apoptosis such as massive chromatin condensation with karyorhexis (Fig. 1, A–C) and PS exposure (Fig. 1C). Accordingly, STS-pulsed cells manifesting PACC had a normal $\Delta V_m$ (as quantified with DiOC$_6$(3)), a low oxidative potential (as quantified with the superanion oxide-reactive dye hydroethidine), failed to manifest the apoptotic activation of Bak (as detected with an antibody that recognizes its exposed N terminus), and showed no sign of mitochondrial cytochrome $c$ release and caspase-3 activation (as determined with an antibody recognizing the neomycin epitope formed by its proteolytic maturation) (Fig. 2). Similarly, AIF was still localized in mitochondria at this stage (38), and RNA interference of AIF did not prevent PACC (not shown). PACC was not prevented by...
the addition of the broad spectrum caspase inhibitor Z-VAD-fmk (Figs. 1, A–D, and 2, A and B). As an internal control of its efficiency, however, Z-VAD-fmk did prevent the advancement to nuclear apoptosis (Fig. 1, A and D), caspase-3 activation, and oxidative reactions (Fig. 2, A and B). PACC was not inhibited by structurally related, more specific caspase inhibitors containing the peptide sequencesVDVAD (specific for caspase-2), DEVD (specific for caspase-3), or LEHD (specific for caspase-9) (not shown). Altogether these data indicate that STS can trigger a morphological state of PACC, which becomes apparent before mitochondrial apoptosis and caspase activation.

**Biochemical Characteristics of PACC—**PACC was not associated with oligonucleosomal DNA fragmentation (which would give rise to TUNEL positivity, Fig. 1D) nor with large scale DNA fragmentation to -50 kbp (Fig. 3A), which can be mediated by caspase-independent death effectors such as AIF (24). However, PACC was accompanied by DNA double strand breaks, as detectable with the sensitive comet assay (Fig. 3, C and D). DNA double strand breaks acutely detectable after the STS pulse were not prevented by Z-VAD-fmk, and the caspase inhibitor did not stimulate their repair upon reculture in STS-free medium (Fig. 3B). As compared with apoptotic nuclei, the transcriptional activity of PACC nuclei was only partially reduced, thus excluding that massive DNA damage had occurred (Fig. 4A). The partial inhibition of transcription by PACC was not specific, as reporter genes indicating the activation of a variety of different transcription factors (NF-kB, AP1, cAMP-response element-binding protein, Stat, p53) were inhibited to a similar level (Fig. 4B). DNA double strand breaks can give rise to the formation of “foci” to which DNA repair enzymes, phosphorylated histone H2AX, and phosphorylated checkpoint kinase-2 are recruited (39, 40). Accordingly, PACC elicited by an STS pulse was accompanied by the formation of nuclear foci containing immunodetectable phosphorylated histone H2AX and phosphorylated checkpoint kinase-2. These foci developed in a caspase-independent fashion and disappeared when PACC advanced to nuclear apoptosis and the nuclear content was degraded (Fig. 5). However, they were maintained in the presence of Z-VAD-fmk. These data suggest that STS causes DNA damage before it induces apoptosis.

**PACC Occurs Upstream of the Mitochondrial Checkpoint—**Bcl-2 and the unrelated vMIA (from cytomegalovirus) can prevent MMP (28, 41). However, neither Bcl-2 nor vMIA did prevent the induction of PACC by short term exposure to STS. They did, however, prevent the advancement from PACC to nuclear apoptosis (Fig. 6A). Moreover, at difference with Z-VAD-fmk (which fails to revert PACC, Figs. 1A and 2), Bcl-2, and in particular, vMIA, did reduce the percentage of cells with PACC observable after overnight culture (Fig. 6A). When Bcl-2 and vMIA prevented the advancement from PACC to nuclear apoptosis (Fig. 6B), they also had a partial inhibitory effect on the apoptosis-associated ΔΨm loss (Fig. 6C), cytochrome c release (Fig. 6D), and caspase-3 activation (Fig. 6E). Importantly, vMIA and Bcl-2 also reduced the percentage of comet-positive cells (Fig. 6F), which is again different from Z-VAD-fmk (which fails to revert comet positivity after PACC, Fig. 3B). Correlating with these results, vMIA and Bcl-2 allowed for the reestablishment of transcription (Fig. 6G), whereas Z-VAD-fmk had no such effect (Fig. 4A). A mitochondrion-targeted Bcl-2 mutant (Bcl-2ActA) also inhibited the advancement of PACC to nuclear apoptosis (Fig. 7A) and the accompanying ΔΨm loss (Fig. 7B), whereas an endoplasmic reticulum-targeted Bcl-2 mutant (Bcl-2cb5) had no such inhibitory effect (Fig. 7, A and B). The reduction of PACC and comet positivity stimulated by vMIA and Bcl-2 depended on expression of the DNA repair enzyme ATM, as shown by the siRNA-mediated knock-down of ATM (Fig. 8, A and B). As a side observation, ATM was required for the phosphorylation of histone H2AX (on Ser-139) and p53 (on Ser-15) yet dispensable for the STS-induced chromatin condensation as such (Fig. 8C). Thus, the reversal of PACC involves ATM-dependent DNA repair. Similar results were obtained when STS was replaced by other agents causing PACC. Thus, Bcl-2 and vMIA did not prevent PACC induced by a short term exposure to the RNA polymerase-γ inhibitor actinomycin D (3 h) (Fig. 9A) or by the DNA-alkylating agent cisplatin (6 h) (Fig. 9B). Again, Bcl-2 and vMIA largely suppressed nuclear apoptosis developing during the subsequent 18 h and actually reversed PACC. Z-VAD-fmk inhibited the progression from PACC to apoptosis yet did not stimulate the reconstitution ad integrum with the loss of PACC (Fig. 9, A and B). PACC could
PACC with a short pulse of STS (vMIA were subjected to an siRNA-mediated knock down of ATM or with the neomycin resistance vector only (neomycin (depends on the expression of ATM. p53 to apoptosis, induced by overnight culture, was reduced in PACC as control cells. However, the advancement from PACC by homologous recombination developed a similar degree of the p53 status because cells in which the p53 has been removed (Fig. 8A) and the conservation of PACC. Importantly, the MMP inhibitors Bcl-2 and vMIA stimulated the repair of the DNA lesions, meaning reversal of PACC (Fig. 6A) and the disappearance of comet assay-detectable DNA breaks (Fig. 6F).

We found it somehow surprising that standard apoptosis-inducing agents such as STS (and actinomycin D) can cause PACC with signs of DNA damage, detectable by comet assay (Figs. 3, 6A) and the conservation of PACC. Importantly, the MMP inhibitors Bcl-2 (including mitochondrion-targeted Bcl-2) and vMIA had a larger range of effects than Z-VAD-fmk (and related inhibitors) suppressed apoptosis when added after PACC induction. However, Z-VAD-fmk failed to reverse the inhibition of transcription and did not stimulate the repair of DNA double strand breaks (Fig. 3, C and D), correlating with the conservation of PACC. Importantly, the MMP inhibitors Bcl-2 (including mitochondrion-targeted Bcl-2) and vMIA had a larger range of effects than Z-VAD-fmk (Figs. 7 and 8). In addition to inhibiting apoptosis, Bcl-2 and vMIA stimulated the repair of the DNA lesions, meaning reversal of PACC (Fig. 6A) and the disappearance of comet assay-detectable DNA breaks (Fig. 6F).

Concluding Remarks—STS, which constitutes a sort of panacea for apoptosis induction (12), can kill cells without a nucleus (9–11). Unexpectedly, however, it appears that a short pulse of high dose STS can trigger double strand breaks (Fig. 3C) correlating with the formation of phosphorylated H2AX foci (Fig. 5A) that also contain phosphorylated Chk2 (Fig. 5A) and p53 (Fig. 8C) and a general yet partial decrease in transcription (Fig. 4), as well as a minor degree of chromatin condensation that occurs before the commonly accepted features of apoptosis become detectable (Fig. 1, A and B). This phenomenon, which we nicknamed PACC, is followed by clear-cut signs of apoptosis when STS is removed from the system: mitochondrial cytochrome c release (Figs. 2 and 6D), caspase activation (Figs. 2 and 6E), PS exposure (Fig. 1C), increased plasma membrane permeability (Fig. 1C), DNA fragmentation into ~50 kbp fragments or shorter (Fig. 3, A and B), TUNEL positivity (Fig. 1D), and total inhibition of transcription (Fig. 4A). PACC can be induced in different cell lines and by different apoptosis inducers (Figs. 9 and 10). The progression of PACC to nuclear apoptosis depends, at least partially, on p53; however, the manifestation of PACC itself is p53-independent, at least in HCT116 cells (Fig. 10). Although this implication of p53 might suggest that the nuclear DNA damage itself might be required for STS-induced apoptosis, published data (9–11) as well as our own data (not shown) indicate that STS can induce apoptosis in cytoplasts, which is in the absence of the nucleus. Thus, the observed PACC is likely to constitute a byproduct of the cytoplasmic effects of STS on multiple kinases, and p53 might mediate part of its impact through its recently described cytoplasmic effects (42, 43). Although PACC itself is not a critical event in STS-induced apoptosis, PACC allows us to stage the STS-induced cellular alterations, while revealing the hitherto unsuspected capacity of STS to induce DNA lesions. Moreover, the way how PACC-associated DNA lesions are repaired may have important implications for the relationship between apoptosis regulation and genomic instability (44). Caspase or MMP inhibitors did not affect the advent of PACC yet did affect the evolution of PACC toward apoptosis. The general caspase inhibitor Z-VAD-fmk (and related inhibitors) suppressed apoptosis when added after PACC induction. However, Z-VAD-fmk failed to reverse the inhibition of transcription and did not stimulate the repair of DNA double strand breaks (Fig. 3, C and D), correlating with the conservation of PACC. Importantly, the MMP inhibitors Bcl-2 (including mitochondrion-targeted Bcl-2) and vMIA had a larger range of effects than Z-VAD-fmk (Figs. 7 and 8). In addition to inhibiting apoptosis, Bcl-2 and vMIA stimulated the repair of the DNA lesions, meaning reversal of PACC (Fig. 6A) and the disappearance of comet assay-detectable DNA breaks (Fig. 6F).

We found it somehow surprising that standard apoptosis-inducing agents such as STS (and actinomycin D) can cause PACC with signs of DNA damage, detectable by comet assay (Figs. 3, C and D, and 8B) and by the formation of foci containing phosphorylated H2AX and Chk2 (Fig. 5A). One of the key observations of this study is that PACC and associated double strand breaks respond differently to caspase and MMP inhibition. Although DNA lesions are “frozen” when cells are cultured with Z-VAD-fmk, meaning that they are not repaired (and eventually lead to cell death or cytogenetic alterations), they disappear in the presence of Bcl-2 or vMIA (Figs. 6–9), provided that the DNA repair machinery is functional (Fig. 8). This may have important consequences for genomic stability because Bcl-2 overexpression can reduce the RAD51-dependent gene conversion and error-free DNA repair (45), thereby causing chromosome aberrations (46). Thus, on theoretical grounds, the repair of double strand breaks stimulated by MMP inhibi-
tors might indirectly increase genomic instability by favoring the persistence of cells that have undergone multiple DNA strand breaks in response to exogenous stress. Irrespective of these theoretical considerations, it appears clear that PACC may occur upstream of the Bcl-2- and vMIA-controlled mitochondrial checkpoint of apoptosis. In the event that MMP is prevented by Bcl-2 or vMIA, PACC-associated double strand breaks can be repaired.

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FIG. 9. Effect of the MMP inhibitors vMIA and Bcl-2 on PACC induced by actinomycin D (A) and cisplatin (B). Cells expressing neomycin (Neo), vMIA, or Bcl-2 were subjected to the indicated treatments, and then the different parameters were assessed (X ± S.D., n = 3; *, p < 0.05; #, p < 0.005 for the calculation of Bcl-2 or vMIA effects as compared with neomycin controls). Note that vMIA and Bcl-2 can cause a reduction of PACC upon overnight reculture, both in the absence and in the presence of Z-VAD-fmk (zVAD), which alone has no inhibitory effect. PI, propidium iodide.

FIG. 10. Effect of p53 on PACC and its advancement to apoptosis. HCT116 cells with the indicated genotypes were cultured with STS (given as a 3-h pulse) followed (or not) by reculture for 18 h, in the absence or presence of Z-VAD-fmk and PACC as well as apoptotic parameters (NA, ΔΨm loss, positive propidium iodide (PI) staining). Note that the absence of p53 (but not that of p21) can reduce PACC and nuclear apoptosis upon reculture but has no effect on PACC induced by acute exposure to STS. X ± S.D., n = 3; *, p < 0.05; #, p < 0.005 for the calculation of p53 and p21 effects as compared with wild type (WT) controls.
Preapoptotic Chromatin Condensation

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