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DNA Damage During Mitosis Invokes a JNK-Mediated Stress Response That Leads to Cell Death

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ABSTRACT

Mitotic catastrophe is a phenomenon displayed by cells undergoing aberrant mitosis to eliminate cells that fail to repair the errors. Why and how mitotic catastrophe would lead to cell death remains to be resolved and the answer will prove valuable in design of better therapeutic agents that specifically target such cells in mitosis. The antibiotic actinomycin D has been shown to induce chromosomal lesions in lower order organisms as well as in human interphase cells. Relatively few studies have been conducted to elucidate molecular events in the context of mitotic DNA damage. We have previously established a model of mitotic catastrophe in human HeLa cells induced by actinomycin D. Here, we show that actinomycin D induce cellular stress via DNA damage during mitosis. The higher order packing of chromosomes during mitosis might impede efficient DNA repair. γH2AX serves as a marker for DNA repair and active JNK interacts with γH2AX in actinomycin D-treated mitotic extracts. We believe JNK might be in part, responsible for the phosphorylation of H2AX and thereby, facilitate the propagation of a positive signal for cell death, when repair is not achieved. The mitotic cell activates JNK-mediated cell death response that progresses through a caspase cascade downstream of the mitochondria. In the mean time, remaining checkpoint signals may be sufficient to put a restraining hand on entry into anaphase and the cell eventually dies in mitosis. J. Cell. Biochem. 110: 725–731, 2010.

KEY WORDS: MITOSIS; MITOTIC CATASTROPHE; JNK; γH2AX; ACTINOMYCIN D

Less is known on mitotic catastrophe although much effort has been directed towards understanding the molecular mechanisms behind this phenomenon. Defined loosely, mitotic catastrophe is a phenomenon displayed by cells that undergo aberrant mitosis. It has been associated with the abnormal activation of cyclin B/Cdk1 and cells that survived the selection process often emerge with abnormalities in nuclear structure and organization [Castedo et al., 2002, 2004]. It has become apparent in recent years that chemotherapy and radiation induce antiproliferative responses, cell death through mitotic catastrophe being one of them. Failure to arrest cell-cycle progression and eliminate these events can lead to chromosomal instability that is likely to contribute to oncogenesis.

The mitogen-activated protein kinase (MAPK) family member, c-jun N-terminal kinase (JNK1/2) is primarily activated by cytokines and environmental stress. JNK signaling has been implicated in the decision between cell survival and apoptosis. A new JNK substrate has been identified, that is phosphorylated in apoptotic cells—H2AX, a histone H2A variant [Lu et al., 2006]. The site of phosphorylation on H2AX corresponds to a non-canonical site for MAPK phosphorylation. In response to DNA damage, phosphorylated H2AX accumulates at the site of double strand breaks (DSBs), where it is thought to restructure chromatin and assist in the recruitment of DNA repair and signaling factors. Lu et al. [2006] showed that JNK-mediated H2AX phosphorylation may be essential for DNA fragmentation in UV-stimulated cells. However, the bulk of the work was aimed at elucidating DNA damage in interphase cells. Relatively little was understood about the same events in a mitotic context.

Actinomycin D was the first antibiotic to display anticancer properties and have been indicated for treatment of a wide range of tumors, including retinoblastoma, Kaposi's sarcoma ecetera. Actinomycin D is an example of a cell-cycle-specific chemotherapeutic agent. It has been known to cause severe damage to genetic material. One study published four decades ago reported that actinomycin D treatment-induced formation of sticky chromatids, especially at the distal segments that seemed to have difficulties

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separating in anaphase; while separated proximal segments were stretched. When treated cells were grown in drug-free medium for several days, high frequencies of endoduplicated mitotic figures were found. In addition to that, chromosome and chromatid breaks and other aberrations were commonly observed [Pathak et al., 1975]. Another group observed that mitosis was delayed in the presence of actinomycin D in Physarum sp. [Vimala Nair and Indirabai, 1991]. These few, but insightful studies constituted the first evidence that actinomycin D led cells to undergo mitotic catastrophe, a term that was coined much later on in 1986.

We have previously established a model of mitotic catastrophe in the human cervical cancer cell line HeLa induced following treatment with the DNA intercalator actinomycin D [Ho et al., 2008]. In this report, we investigated the cellular stress response to DNA damage introduced by actinomycin D during mitosis, a unique time frame of the cell cycle whereby genetic material is condensed into rigid, tightly packed chromosomes. γH2AX serves as a marker for DNA repair. The higher order packing of chromosomes during mitosis renders the repair mechanism inefficient. The mitotic cell activates JNK-mediated cell death response that progresses through a caspase cascade downstream of the mitochondria.

MATERIALS AND METHODS

ANTIBODIES

Antibodies to Actin, Cyclin B1, and Cdc2 p34 HRP were purchased from Santa Cruz (Beverly, MA). Antibodies to phospho-Histone H3 were from Calbiochem® (San Diego, CA), MAD2 were from Covance (Denver, PA), Tubulin-fluorescein isothiocyanate (FITC) were from Sigma (MO), AIM-1/Aurora B and BubR1 antibodies were from BD Transduction Laboratories (Palo Alto, CA), and Novus Biologicals (Littleton, CO). Cdc20 antibodies were from MBL International. Separase antibodies were from Abcam. γH2AX, H2AX, phospho-Cdc2, Cleaved ICAD/DFF40 antibodies, and JNK Phosphoplus Kit were from Cell Signaling (Danvers, MA). Secondary antibodies conjugated with appropriate fluorophores were purchased from Invitrogen (Carlsbad, CA). Horseradish peroxidase (HRP)-conjugated secondary antibodies were from Dakocytomation (Glostrup, Denmark).

CELL CULTURE AND DRUG TREATMENTS

HeLa cells were maintained in DMEM GlutaMax medium (Gibco, Invitrogen) containing 10% fetal calf serum (Hyclone), and 1% penicillin/streptomycin at 37°C in a humidified atmosphere with 5% carbon dioxide. For drug treatment, HeLa cells were treated with 600 nM nocodazole (Sigma). For actinomycin D (Sigma) treatment, cells were supplemented with 400 nM actinomycin D for the required periods. Time points were collected at 2 h intervals.

IMMUNOFLUORESCENCE STAINING

For immunofluorescence staining, cells were collected and fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and permeabilized with 0.5% Triton X-100 for 5 min at room temperature. Primary antibodies in 4% bovine serum albumin in Tris-buffered saline (TBS) with 0.05% Tween-20 were incubated for 1 h at room temperature or overnight at 4°C. Cells were washed three times and incubated with appropriate secondary antibodies for 1 h at room temperature. Slides were mounted in Prolong Gold Anti-Fade medium with DAPI (Invitrogen). Images were collected and analyzed on an Axiovert 200 M inverted fluorescence microscope (Carl Zeiss, Germany).

WESTERN BLOTTING

Mitotic cells were collected via shake-off and lysed in M-PER Mammalian Lysis Buffer (Pierce, Rockford, IL) and samples were boiled for 5 min before being subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), followed by transfer to nitrocellulose membrane. HRP-conjugated secondary antibodies were from Dakocytomation. Detection was via chemi-luminescence using ECL reagent from GE Healthcare (Little Chalfont, Buckinghamshire, UK) or Thermo-Scientific (Rockford, IL).

IMMUNOPRECIPITATION

HeLa cells were lysed with Pierce M-PER Mammalian Lysis Buffer. Lysates were clarified by centrifugation at 13,200 rpm for 10 min at 4°C. Clarified lysates were incubated with primary antibody at 4°C overnight. Rec. Protein G-Sepharose® 4B (Invitrogen) was then added to the lysates and the mixture was incubated for an additional 2 h. After that, immunoprecipitates were washed five times with wash buffer (1× TBS with 250 mM NaCl) and proteins were recovered by boiling the beads in sample buffer and analyzed by Western blotting.

RESULTS

HIGHLY COMPACTED MITOTIC DNA CAN BE DAMAGED IN THE PRESENCE OF ACTINOMYCIN D

We examined mitotic cells for acquired DNA lesions. HeLa cells were treated with nocodazole and actinomycin D and mitotic cells were collected by mitotic shake-off at the indicated time point in Figure 1A. More than 95% of cells isolated by this method were arrested in mitosis (Supplementary Fig. 1). Mitotic figures prepared from actinomycin D-treated cells showed obvious DNA damage at the chromosomal level such as fragmentation and formation of dicentric chromosomes. Control mitotic chromosomes, on the other hand, were compact and tightly condensed and displayed little spontaneous aberrations (Fig. 1B). Previously, it has been reported that actinomycin D indirectly induces DNA double-strand breaks in interphase chromatin, when a stalled topoisomerase I collides with a replication fork [Mischo et al., 2005]. We showed in this report that this antitumor agent has the ability to induce chromosomal lesions during mitosis, when the chromosomes are rigid and compact.

H2AX IS PERSISTENTLY PHOSPHORYLATED EVEN WHEN THE GENOTOXIC AGENT IS REMOVED

The histone H2A variant, H2AX is a well-known marker of double-stranded DNA breaks [Rogakou et al., 1998; Forand et al., 2004]. H2AX has an extended carboxyl terminal that is characterized by a unique Ser-Gln-Glu (SQE) motif. This sequence in the C terminus of H2AX is a target for post-translational modification during DNA damage, during which serine 139 is phosphorylated, giving rise to the γH2AX moiety. γH2AX accumulates around sites of DNA lesions

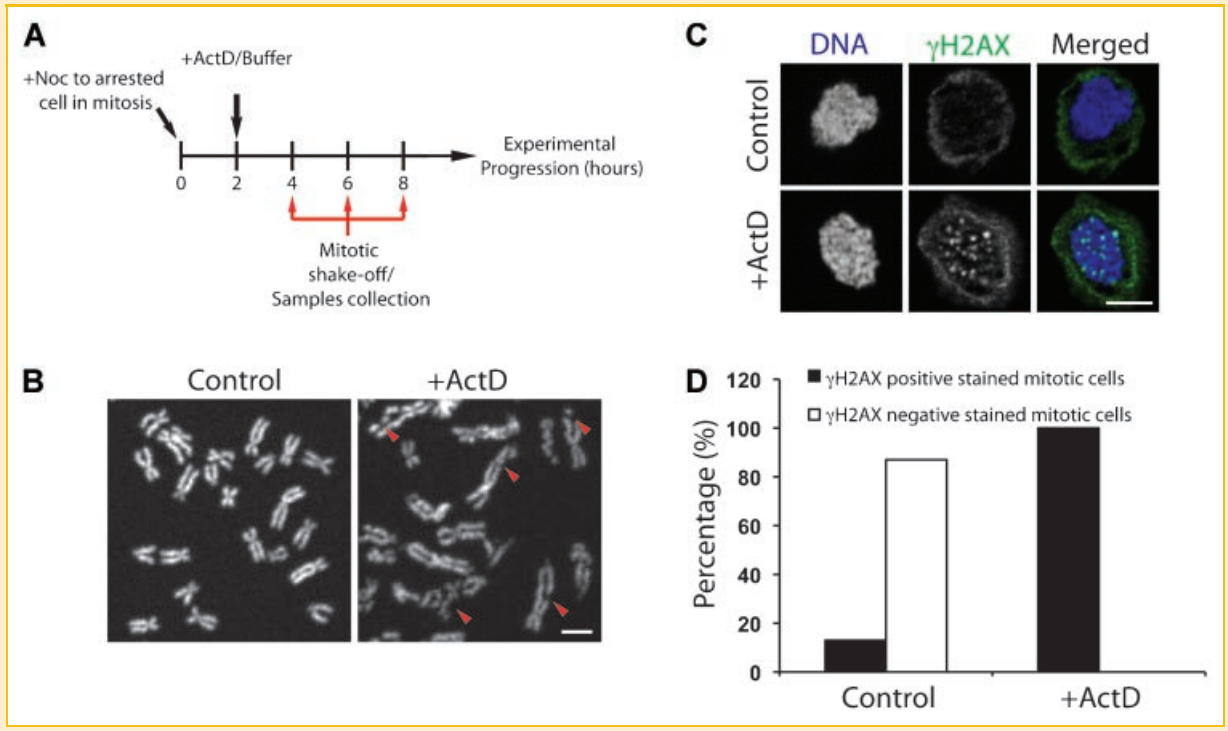

Fig. 1. A: Timeline showing experimental design. Cells were arrested using nocodazole before actinomycin D was introduced. Mitotic cells were collected via shake-off at 2-h intervals. B: Mitotic chromosomes exhibit chromosomal breaks and other aberrations upon treatment with actinomycin D. Bar, 5 μm. C: γH2AX recruitment to discrete foci on chromosomes in mitotic cells damaged by actinomycin D. Representative cells were displayed with DNA in blue and γH2AX in green, respectively. Bar, 10 μm. D: Mitotic cells with ≥3 discrete foci were categorized as γH2AX positive. Cell counting was done in a sample of at least 30 mitotic cells and the data were represented as a bar chart.

and is thought to recruit downstream repair proteins. γH2AX formed distinct foci on the chromosomes in mitotic cells induced to undergo mitotic catastrophe (Fig. 1C). Cells with three brightly stained foci were scored as γH2AX positive. Practically all actinomycin D-treated mitotic cells (100%) were decorated with this moiety, compared to ~13% in control mitotic cells (Fig. 1D). The Western blot findings corroborated with immunofluoresence data that γH2AX levels were dramatically upregulated in actinomycin D-treated mitotic lysate (Fig. 2A).

γH2AX facilitates post-replicational DNA repair by recruiting chromatin remodeling complexes at DSB lesions, which have been suggested to decondense chromatin and allowing access for other repair factors [Furuta et al., 2003]. The importance of γH2AX in DNA repair has been implied not only by γH2AX role as the player which kickstarts the DNA repair signaling cascade, but dephosphorylation of γH2AX is required to switch off the checkpoint signal [Nazarov et al., 2003; Chowdhury et al., 2005]. We were interested to know if removal of the death stimuli would furnish a chance for mitotic cells to repair their DNA. Mitotic chromosomes are highly condensed entities and are functionally and structurally different from interphase chromatin. It is therefore intriguing to think of how repair factors might get about performing their function in a compact environment. Cells were first arrested in mitosis before addition of actinomycin D, which was withdrawn after 2 h of treatment. Fresh growth medium with nocodazole was provided subsequently to keep the cells in mitosis. γH2AX profiles were similar to that obtained earlier (Fig. 2B,C). This data therefore suggest that the repair pathway was not efficient when the cells were maintained in mitosis, which might be partly due to the compact organization of mitotic chromosomes.

JNK PATHWAY IS ACTIVATED AND TARGETS H2AX IN VIVO

The MAPKs are a family of proteins mediating distinct signaling cascades that are targets for a myriad of extracellular stimuli. It has been reported in the literature that JNK or stress-activated protein kinase (SAPK) is activated in response to a variety of stress factors. JNK activation is mediated by dual phosphorylation on Thr and Tyr residues by the MAPK kinases MKK4 and MKK7 [Ip and Davis, 1998]. It was previously reported that duration of JNK phosphorylation decides whether the outcome is cell-cycle regulation or apoptosis [Chen et al., 1996].

JNK was phosphorylated in actinomycin D-treated mitotic cells but not in control cells (Fig. 3B). We also observed that activation of JNK coincides with phosphorylation of histone H2AX and we proceeded to check if the two events are related. Although members of the PI3K family, including ATM, ATR, and DNA-PK were reported to be involved in mammalian DNA damage response, there is no direct data showing that these kinases phosphorylates H2AX in vivo [Burma et al., 2001; Ward and Chen, 2001; Bakkenist and Kastan, 2003; Park et al., 2003; Ward et al., 2004; Lu et al., 2006]. Furthermore, formation of γH2AX was observed in cell lines deficient in one or three of the PI3Ks [Fernandez-Capetillo et al., 2002; Koike et al., 2007]. JNK was previously proposed and proven to be the kinase responsible for phosphorylating the histone variant

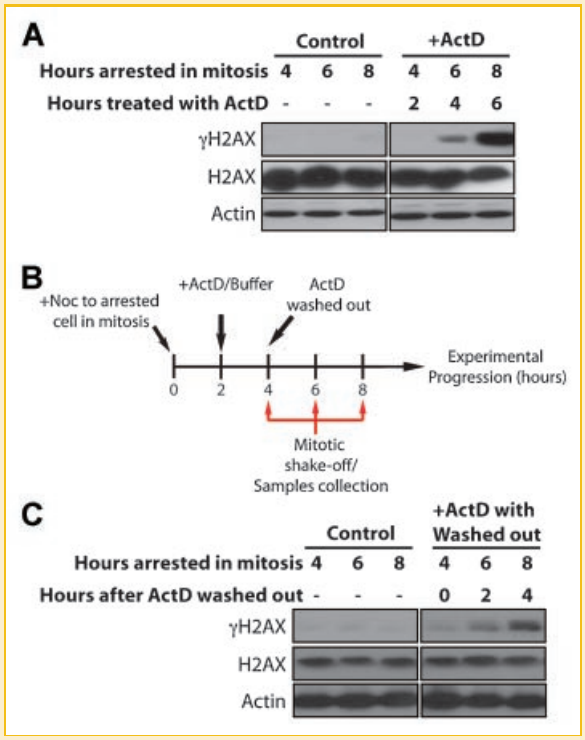

Fig. 2. A: γH2AX expression levels accumulated in actinomycin D-treated mitotic cells over time but not in control mitotic cells. B: Timeline showing experimental design. Cells were arrested in mitosis using nocodazole. Subsequently, actinomycin D was added for 2 h before washing out. Cells were collected in the continued presence of mitotic block via mitotic shake-off at 2-h intervals. C: Gamma H2AX expression persists even though actinomycin D had been removed.

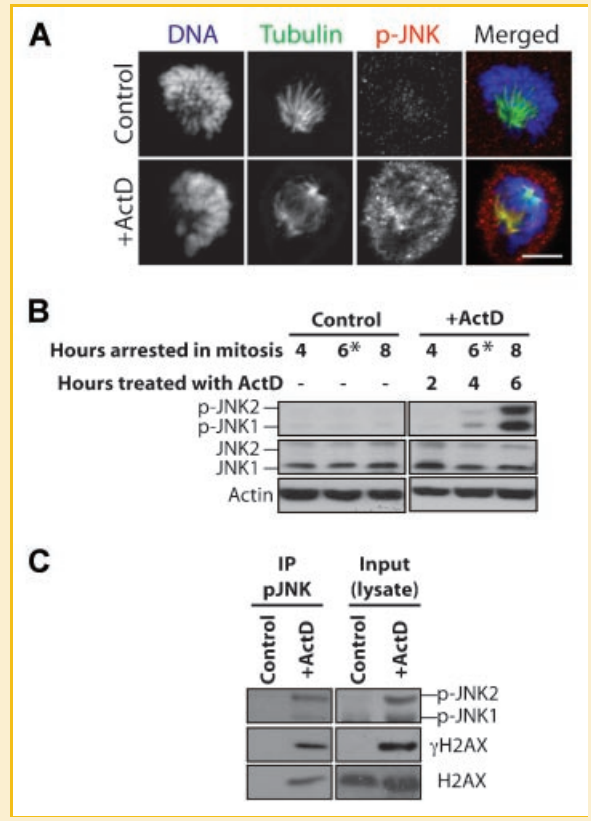

Fig. 3. A: Phosphorylated JNK co-localized onto chromosomes and partially with the spindle apparatus. Cells were treated as illustrated in Figure 1A and immunofluorescence staining was done using antibodies against microtubules (green) and phosphorylated JNK (red). Samples were counter-stained with DAPI. Bar, 10 μm. B: Mitotic cells treated with actinomycin D showed activation of JNK1/2 over time, but not in control. C: Phosphorylated JNK co-immunoprecipitated γH2AX in actinomycin D-treated mitotic lysate but not in control lysate. Mitotic cells were collected 6 h (*) after of mitotic arrest by mitotic shake-off and subjected to immunoprecipitation with 4 μg of anti-pJNK antibody and probed for γH2AX and H2AX, respectively.

H2AX to form γH2AX in an array of in vitro and in vivo assays [Lu et al., 2006]. We did a pulldown using pJNK antibody and indeed, γH2AX was precipitated together with pJNK in actinomycin D-treated mitotic lysate (Fig. 3C) but not in control. This indicated physical interaction between pJNK and γH2AX and that JNK is a potential candidate as kinase for histone H2AX in the context of actinomycin D-triggered mitotic catastrophe.

The induction of γH2AX foci in actinomycin D-treated cells demonstrated that DNA damage could occur in highly condensed DNA. It is a possibility that the signal for DNA damage is amplified by γH2AX foci, which was created by the activity of phosphorylated JNK. If so, JNK must localize to chromosomes for it to be of close proximity with its target substrate. JNK is associated with centrosomes throughout the cell cycle and is largely found in the cytoplasm relative to the nucleus [MacCorkle-Chosnek et al., 2001]. We co-stained for phosphorylated JNK and spindle fibers after drug treatment. In actinomycin D-treated cells, we observed phosphorylated JNK to be localized on chromosomes as well as on the spindle apparatus, but not in control (Fig. 3A). This is reminiscent of the formation of γH2AX foci on damaged DNA.

CASPASE ACTIVATION AND A POSSIBLE MITOCHONDRIAL-LINKED CELL DEATH

SAPKs, as their name implies, is primarily related to the relay of stress signals in apoptotic pathways. However, in a non-stress setting, SAPKs, in particular the JNK pathway, have been suggested to contribute to proliferative responses, as suggested by genetic and biochemical evidences. Current data indicate that signaling through the JNK pathway is important in the activation of the mitochondria-dependent apoptotic pathway (intrinsic pathway) but dispensable for apoptosis induced by activation of the death receptors (extrinsic pathway).

We asked whether mitotic catastrophe displays the most prominent biochemical apoptotic hallmark—caspase activation. Caspases execute the cell's decision to undergo apoptosis. Caspases are activated proteolytically and upstream-activated caspases cleave their downstream counterparts. Our data showed mitotic catastrophe to be dependent upon caspase activation. Mitotic cells treated with the DNA-damaging agent exhibited cleaved patterns of all the effector caspases, including caspases 3, 6, and 7. The downstream effector caspases cleave key proteins involved in apoptosis and they play non-redundant roles in the execution of apoptosis. Caspases 6 and 7 have been suggested to compensate for

the lack of caspase 3. For example, MCF-7 cells, which lack caspase 3, undergo mitochondrial-dependent apoptosis via caspase 7 activation [Twiddy et al., 2006]. Moreover, caspase 9, a constituent that makes up the apoptosome together with APAF-1 and cytochrome *c* to cleave caspase 3 downstream of the mitochondria in response to apoptotic signals is also activated (Fig. 4A). Assembly of the apoptosome leads to processing of procaspase 9 to yield the large and small subunits of caspase 9. Our data hints at a possible involvement of the intrinsic pathway via release of mitochondrial proteins. Control mitotic cells arrested with nocodazole did not show activation of these apoptotic parameters, ruling out the possibility that mitotically arrested cells display activation of these apoptotic variables per se.

INHIBITOR OF CASPASE-ACTIVATED DNase, DFF45 IS CLEAVED

DNA fragmentation factor (DFF) (human homologue of murine ICAD) is a heterodimeric protein composed of 45-kDa (DFF45) and 40-kDa (DFF40) subunits, a protein that mediates regulated DNA fragmentation and chromatin condensation in response to apoptotic signals [Widlak, 2000]. DFF45 is a specific molecular chaperone and an inhibitor for the nuclease activity of DFF40. Previous studies have shown that upon cleavage of DFF45 by caspase 3, the nuclease activity of DFF40 is relieved of inhibition [Liu et al., 1999]. The cleaved DFF45 fragments dissociate from DFF40, allowing DFF40 to oligomerize to form a large functional complex that cleaves DNA by introducing DSBs [Gu et al., 1999; Inohara et al., 1999].

DFF40 in UV-treated cells cleaved DNA strongly when H2AX was phosphorylated. Without H2AX phosphorylation, caspase 3/CAD (DFF40) cannot induce DNA fragmentation [Lu et al., 2006]. Cleaved DFF45 accumulated in actinomycin D-treated mitotic cells over time but not in control (Fig. 4A). Thus, this gives us an indication that the function of γH2AX might not be restricted to DNA damage repair but could play a part in regulation of apoptosis.

SUPPRESSION OF HISTONE H3 AND Cdc2 PHOSPHORYLATION UPON DNA DAMAGE

Phosphorylation of histone H3 is a marker of mitotic cells. Phosphorylation of histone H3 at Ser10 usually begins before prophase. This modification is added by chromosome passenger protein Aurora B kinase [Goto et al., 2002; Sugiyama et al., 2002]. The level of phosphorylated histone H3 increased in control mitotic cells treated with nocodazole with increasing time but remained at basal level in cells exposed to actinomycin D. Cdc2 activation is another indication of the cell's mitotic status. We observed that upon actinomycin D treatment, these mitotic cells lost their Cdc2 phosphorylation signature that was needed to form the active mitosis promoting factor (MPF) (Fig. 4B,C). Taken together, they indicate the mitotic cells are attempting to exit mitosis.

MITOTIC CHECKPOINT COMPLEX IS DISRUPTED

We wondered whether DNA damage would affect the spindle checkpoint components as it has been shown that an active spindle checkpoint is required for mitotic cell death induced by DNA damage [Nitta et al., 2004]. BubR1 and Mad2 are crucial members that constitute the spindle checkpoint complex, and activation of which serves to keep the anaphase promoting complex and its activator, Cdc20 in check. The mitotic checkpoint ensures accurate chromosome segregation during mitosis. Mutations in genes encoding essential checkpoint proteins such as BUB and MAD lead to chromosomal instability and promote cancer development [Kops

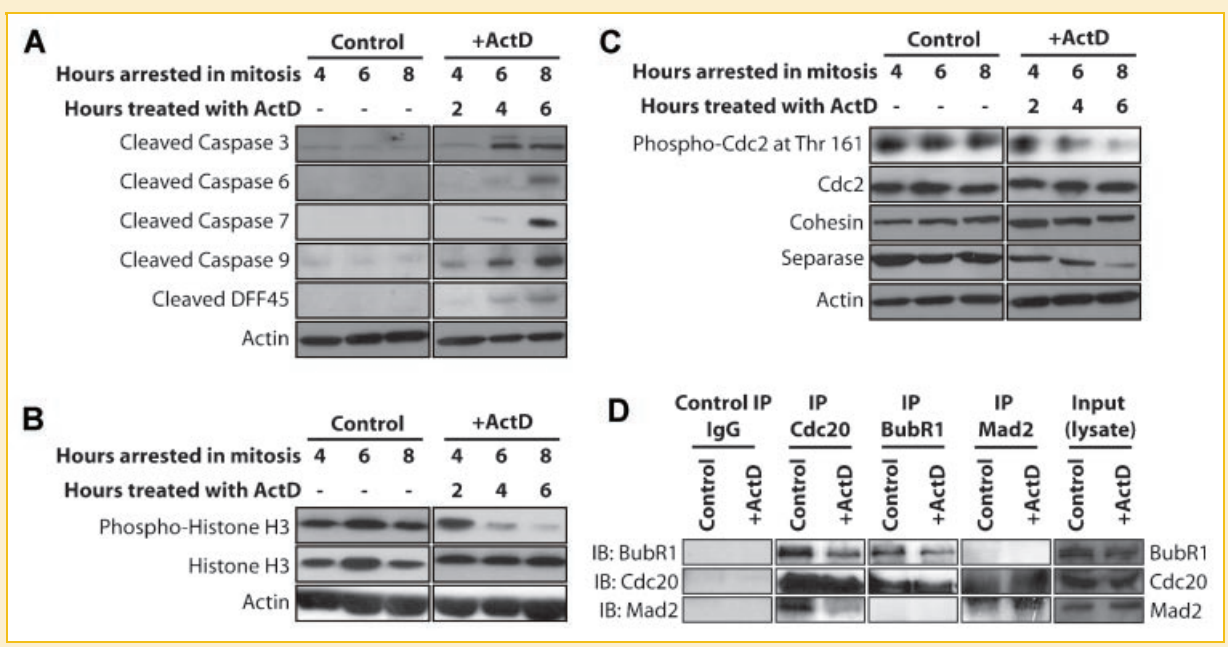

Fig. 4. A: Caspase cascades are activated in actinomycin D-treated mitotic cells, but not in control. Cell death occurs via a mitochondria-related pathway. Caspase substrate DFF45 was also cleaved. B: Histone H3 phosphorylation is suppressed in mitotic cells in the presence of actinomycin D. C: Levels of various mitotic regulators were checked in actinomycin D-treated mitotic cells. Cdc2 was dephosphorylated and separase cleavage occurred. D: Interactions within the mitotic checkpoint complex was disrupted. Immunoprecipitation reactions were done using 1.5 mg/ml mitotic lysate. Forty micrograms of lysate were loaded to check for protein expression.

et al., 2005]. BUB and MAD are negative regulators of the Anaphase Promoting Complex/Cyclosome (APC/C) during mitosis to prevent cells with unaligned chromosomes from entering anaphase prematurely.

We have performed endogenous immunoprecipitations of components of the mitotic checkpoint complex. Briefly, mitotic cells were collected after the relevant treatments and subjected to immunoprecipitation by various antibodies against Mad2, BubR1, and Cdc20, respectively. Cdc20 association with phosphorylated form of BubR1 appeared to be reduced in actinomycin D-treated mitotic cells. Reciprocal immunoprecipitations with a BubR1 antibody showed consistent result. It was noted that the BubR1 antibody did not pick up Mad2 as an interaction partner of BubR1. These data lend support to the hypothesis that there exists diffusible complexes of the mitotic checkpoint that is not dependent on kinetochore localization and this, in conjunction with our previous immunofluorescence data [Ho et al., 2008], could imply that the diffusible and kinetochore checkpoint complexes might complement each other's activity [Fang, 2002]. Meanwhile, interactions between freely diffusible Cdc20 and Mad2 did not demonstrate any major changes (Fig. 4D). All in all, these data further substantiate a weakened but still functional checkpoint signal and cooperative interaction between the two arms of the spindle checkpoint to maintain mitotic arrest preceding catastrophic cell death.

CLEAVAGE OF ANAPHASE REGULATOR SEPARASE

Separase initiates sister chromatid separation by cleaving the Scc1 subunit of cohesin and thereby resolving the glue that holds sister chromatids together. Activation of separase requires degradation of its inhibitor securin and removal of inhibitory phosphates [Holland and Taylor, 2006]. In human cells, separase activation at anaphase onset not only coincides with securin degradation and cohesin cleavage but also with cleavage of separase itself. It was shown that securin inhibits separase by blocking access of substrates to the active site of separase and securin degradation induces separase to undergo autocatalytic cleavage at one of three potential sites. However, separase cleavage is not essential for its activation [Waizenegger et al., 2002]. Separase was noted to undergo cleavage, possibly via autocatalytic cleavage (Fig. 4C). However, from our mitotic figures, sister chromatid pairs did not appear to have undergone separation (Fig. 1B). A recent publication showed an additional mechanism keeps separase inactive in Xenopus egg extracts displaying high Cdc2 activity. Inhibitory phosphorylation at a specific site functions independently of securin binding and complete activation of separase is proposed to require both securin removal and separase dephosphorylation [Stemmann et al., 2001; Huang et al., 2005]. Hence, arrested cells did not appear to have experienced anaphase or mitotic slippage prior to mitotic catastrophe.

DISCUSSION

In this report, we explored whether DNA breakage caused by actinomycin D is reparable during mitosis and we are interested in the downstream events incurred after detection of DNA lesions. Understanding how DNA damage can lead to cell death will provide valuable information in the design of better therapeutic agents.

γH2AX foci label damaged sites on DNA. It is widely accepted that γH2AX foci recruit DSB repair proteins and dissolve when repair is completed. Actinomycin D-treated mitotic cells exhibited chromosomal abnormalities that accumulated over time. In a mitotic cell, the chromosomes are packed tightly into a highly compacted structure. DNA repair factors may have a tougher time to access damaged sites. Hence, homologous recombinational repair or even DNA end-joining cannot occur efficiently. γH2AX levels increased and persisted, regardless of whether the damaging stimuli was maintained or removed from cells, indicating inability to repair the damage and to switch off the positive signal for downstream death signaling. The continuous propagation of this signal may lead to activation of downstream caspase-related cascades.

When cytotoxic drugs were first generated, they were believed to mainly target cancer cells because of their rapid proliferation rate. Today, we understand this view is simplistic and inadequate to explain the complex cellular processes that drive tumorigenesis. Cytotoxic agents owe their efficacy not only to the fact that they cause cellular injury directly, but also because of the stress response being set into motion as a secondary effect of the insult afflicted upon genetic material or cytoskeleton.

We have investigated the effect of MAPKs on the cellular decision to undergo catastrophic cell death. MAPKs exert broad range of effects on cellular activities. Among them, SAPK/JNK was activated by stress imposed on cells. The exact effects of JNK on cellular events have not been fully elucidated although JNK1 is mainly associated with the induction of apoptosis while JNK2 seems to have an effect on anaphase progression. Mitotic cells appear more "potentiated" than cells in other phases of the cell cycle in the sense that they have higher sensitivity towards stress stimuli. JNK phosphorylates H2AX at a non-canonical site for MAPKs. We show that active JNK co-immunoprecipitates γH2AX in actinomycin D-treated mitotic extracts. It is tempting to speculate that JNK might be in part, responsible for the phosphorylation of H2AX and thereby, facilitate the propagation of a positive signal for cell death, when repair is not achieved.

In our model of cell death, cells try to exit mitosis by attempts to switch off the mitotic spindle checkpoint. However, we hypothesized that a functional checkpoint signal carried by both kinetochore-dependent and -independent complexes exist, complying with the notion that the spindle checkpoint is a pre-requisite to drive the cells toward mitotic catastrophe. Events such as dephosphorylation of histone H3 and Cdc2 as well as degradation of anaphase inhibitor separase point toward an attempt to exit mitosis. Histone H3 has been postulated to adopt a more closed configuration when it is phosphorylated on the N terminal tail as the negative charge on the phosphate group neutralizes the positive charge on the N terminal lysines. Dephosphorylation of histone H3 brings about global decondensation of chromosomes, which, is assumed to be a more practical setting for cells to repair damaged DNA.

Hereby, we propose a model of mitotic catastrophe in HeLa cells induced by actinomycin D whereby persistent DNA damage invoked stress responses mediated by the SAPK/JNK. The signal relayed by γH2AX amplifies the initial insult and drives the cell to activate

downstream apoptotic pathways by the intrinsic mechanism. The cell attempts to exit mitosis as a default setting in the event of DNA damage, as uncondensed, relaxed chromatin is more accessible for repair factors. However, the remaining checkpoint signal carried by kinetochore-dependent and -independent mechanisms appear to be sufficient to put a restraining hand on entry into anaphase and the cell eventually dies in mitosis.

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