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Novel DNA damage checkpoint in mitosis: Mitotic DNA damage induces re-replication without cell division in various cancer cells

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ABSTRACT

DNA damage induces multiple checkpoint pathways to arrest cell cycle progression until damage is repaired. In our previous reports, when DNA damage occurred in prometaphase, cells were accumulated in 4 N-DNA G1 phase, and mitosis-specific kinases were inactivated in dependent on ATM/Chk1 after a short incubation for repair. We investigated whether or not mitotic DNA damage causes cells to skip-over late mitotic periods under prolonged incubation in a time-lapse study. 4 N-DNA-damaged cells re-replicated without cell division and accumulated in 8 N-DNA content, and the activities of apoptotic factors were increased. The inhibition of DNA replication reduced the 8 N-DNA cell population dramatically. Induction of replication without cell division was not observed upon depletion of Chk1 or ATM. Finally, mitotic DNA damage induces mitotic slippage and that cells enter G1 phase with 4 N-DNA content and then DNA replication is occurred to 8 N-DNA content before completion of mitosis in the ATM/Chk1-dependent manner, followed by caspase-dependent apoptosis during long-term repair.

1. Introduction

The cells possess a defense system called checkpoints to resist from DNA damage and genotoxic insults, which are present at the G1/S boundary, intra-S, and G2/M transitions [1,2]. In the event of DNA damage, sensor proteins and signal transducer proteins associated with each checkpoint detect DNA damage and transmit signals to their appropriate effectors, which initiate cell cycle arrest, DNA repair, or apoptosis. When repair of DNA damage is completed, the cells restart the cell cycle. If cells do not successfully complete DNA repair, the cells continue cell cycle arrest and then must be removed by apoptosis or cellular senescence or oncogenesis [3,4]. The ataxia-telangiectasia mutated (ATM) activates checkpoints by transmitting a DNA damage transduction signal when cells are exposed to ionizing radiation or drugs that trigger DNA double strand breaks [5]. The ataxia-telangiectasia and Rad3-related (ATR) is known to be activated by stalled replication forks and UV-radiation-induced DNA damage [6,7]. ATM/ATR transmits actual signals to major downstream targets, such as Chk1 and Chk2 [3]. These checkpoint kinases negatively regulate Cdc25 phosphatase family proteins that dephosphorylate Cdks involved in the cell cycle transition [8]. Cdk1 activity and its destruction are required for regulation of the mitotic exit, proper chromosome segregation, cytokinesis, and progression to G1 phase in the normal cell cycle. Although the activation status of Cdk1 is dependent upon cyclin B1 binding, the tyrosine-15 phosphorylation site of Cdk1 is regulated by the Cdc25 phosphatase family through the ATM-Chk1/2-Cdc25C pathway [9,10]. Plk1 is associated with phosphorylation of Cdc25 and mitotic cyclin, centrosome maturation [11], establishment of bipolar spindle [12], and activity of the anaphase-promoting complex (APC/C) [13]. Plk1 is also a target of DNA damage checkpoint in an ATM-dependent manner [14,15]. Plk1 inhibition in this situation leads to cell cycle arrest [16,17]. In addition, mitotic DNA damage delays mitotic exit in association with inhibition of Cdk1 and Plk1, cytokinesis failure, and abnormal G1 phase with 4N DNA content, even cyclin B1 level is still high [16–18]. These cells have decreased phosphorylation of Cdc25C compared with mitotic phase cells [19]. DNA damage may generate an abnormal DNA phenotype in mitosis called multiploidy. Multiploidy induces chromosomal instability and is a common feature of tumor cells. It may promote tumor formation with a combination of additional factors in tissue or act to protect organisms from tumor formation by cell death [18,20]. In this report, we showed that mitotic DNA damage induced abnormal cell cycle arrest in G1 phase with 4N DNA content without cell division in the ATM/Chk1-dependent manner, and multiploidy followed to DNA re-replication without completion of mitosis was observed.

2. Method and materials

2.1. Cell culture and treatments

Cells were maintained in Dulbecco's modified Eagle's medium containing 10% FBS (Hyclone). To synchronize in prometaphase,

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cells were treated with nocodazole (100 ng/ml, Sigma) for 16 h and collected by shake-off. For mitotic DNA damage, mitotic cells were treated with doxorubicin (5 μM, Sigma) for 1 h. Cells were incubated in fresh media for the indicated time. For inhibition of DNA replication, mitotic cells with doxorubicin shock were treated with hydroxyurea (2 mM, Sigma) for 24 h. To detect BrdU incorporation, cells were treated with bromodeoxyuridine (BrdU) (10 μM, Sigma). To address caspase dependency, mitotic cells were treated with Z-VAD-FMK (100 μM, R&D system) for 2 h prior to doxorubicin shock, and continuously incubated with Z-VAD-FMK (10 μM) during releasing.

2.2. Cell transfection, gene silencing, and overexpression

Cells were transfected with plasmid DNAs by the calcium chloride technique. Plasmid construct of short hairpin RNA (shRNA) for gene silencing were provided by SABiosciences (Frederick, MD, USA). SureSilencing™ shRNA plasmid provided by SABiosciences (Frederick, MD, USA) expressed was used for gene-specific knockdown. Target sequences for knockdown of human Chk1 and ATM genes was as follow: 5′-1TTG GTT GAC TTC CGG CTT TCT-3′ and 5′-CCA GAA TGT GAA CAC CAC CAA-3′, respectively.

2.3. Analysis of cell cycle and BrdU incorporation by flow cytometry

Cells were trypsinized, fixed in ice-cold 80% ethanol for 16 h or longer, and incubated with RNaseA (100 μg/ml) at 37 °C for 2 h. Cells labeled by propidium iodide (40 μg/ml) were analyzed by flow cytometry of 25,000 events (FACSCaliber, Becton Dickinson). For quantification of BrdU incorporation, cells labeled with BrdU were harvested and fixed in ice-cold 70% ethanol for 16 h or longer. Cells were treated with 1 ml of HCl (1.5 M) at RT for 20 min, and incubated for 1 h in PBS containing 0.5% Tween-20, 0.5% BSA, and anti-Brdu antibody (1:200 dilution, SantaCruz). Cells were incubated with FITC-conjugated secondary anti-mouse antibody (1:100 dilution, SantaCruz) and propidium iodide (40 μg/ml).

2.4. Microscopic analysis

For confocal microscopic analysis of BrdU incorporation, cells were cultured on glass coverslips and fixed in 4% paraformaldehyde for 15 min at RT. After washing, cells were incubated in Triton X-100 buffer (0.3% Triton X-100 in PBS) for 15 min at RT. For DNA denaturation, cells were incubated with 2 M HCl for 20 min, and were neutralized using 0.1 M sodium borate for 2 min. Cells were blocked in blocking buffer (10% Tween 20 and 2% horse serum in PBS) for 30 min, and were stained with BrdU conjugated FITC antibodies. The fluorescence signals were detected and captured by confocal microscopy (LSM510). For time-lapse microscopic analysis, mitotic cells were grown in glass bottom dish and detected on a microscope (Zeiss Axiovert S100) equipped with a LCI long-term microincubator chamber system with CO_2.

2.5. Western Blotting and antibodies

For Western Blot analysis, cells were lysed in NP-40 cell lysis buffer (0.5% Nonidet P-40, 150 mM NaCl, 20 mM Tris–HCl, pH 8, 2 mM EDTA, pH 8.0, 2 mM EGTA, pH 8.0, and protease inhibitors). Cell lysates were separated on SDS–PAGE. Proteins were transferred to a polyvinylidene difluoride membrane (Millipore). Following incubation with antibodies in TBS containing 0.05% Tween-20 and 5% skim-milk, protein signals were visualized using the ECL™ system (Amersham Biosciences). Antibodies for Chk1, ATM and PARP-1 were obtained from Santa Cruz Biotechnology. Anti-Caspase-3 antibodies were obtained from Cell Signaling.

3. Results

3.1. Mitotic HeLa cells with DNA damage do not undergo cytokinesis and enter G1 with 4 N-DNA content, and re-replication of DNA occurs without cell division

We previously reported that prometaphasic cells accumulate in G2-like interphase and not in mitosis as a result of DNA damage by doxorubicin shock [17]. Under this condition, activated Cdk1 in prometaphase became inactivated, and mitotic Plk1 was also dephosphorylated and inhibited by protein phosphatase 2A, which might function as a downstream target of the ATM/Chk1 pathway. Upon inactivation of two major mitotic kinases, cells cannot undergo the remaining steps of mitosis; instead, they enter G1 phase with 4N DNA content. These phenotypes of the mitotic DNA damage response were detected from 3 h after release into fresh media for repair [16].

To investigate the fate of mitotic cells with DNA damage after prolonged culture through this novel mitotic DNA damage response, cells were incubated for 24 h or longer. Cells synchronized in prometaphase were treated with doxorubicin for 1 h and then cultured continuously after washing for damage repair (Fig. 1A). During culture, we followed the cell cycle progression, and phenotypic changes in cells were detected by time-lapse imaging (Fig. 1B, C). Most mitotic cells without DNA damage by doxorubicin shock progressed into cytokinesis for further division within 30 min and became two daughter cells in G1 phase (Fig. 1B). After incubation for 13–15 h, cells entered into a new mitotic period and started a second round of cell division. On the other hand, when cells were treated with doxorubicin, they did not enter cytokinesis and remained as a single cell even during prolonged culture. Under this condition, cells with DNA damage turned into enlarged cells with multi-nuclei within 20 h of incubation (Fig. 1C). To distinguish between a defect in chromosomal segregation or nuclear division and a defect in cytokinesis, we performed time-lapse microscopic analysis using fluorescent label on chromosomes by expressing histone H2B-GFP. During releasing for 6 h, mitotic chromosomes were not segregated properly through normal anaphase, and cellular phenotypes of late mitosis were not observed (Fig. 1D, upper panels). As with Fig. 1C, multi-nuclei were observed in mitotic cells with DNA damage within 24 h after release (Fig. 1D, lower panels).

Normal mitotic cells without doxorubicin shock contained 4 N-DNA content, and most of the cells entered a new round of cell division, finally accumulating in interphase with 2 N-DNA content (Fig. 1 , 20 h in a), as in normal asynchronous culture (Fig. 1E, g). When the doxorubicin concentration was increased from 0.2 μM to 5 μM, accumulation of cells in interphase containing 4 N-DNA content within 8 h after release increased (Fig. 1E, 8 h in b–f). After incubation for 20 h or longer, cells with 4 N-DNA content were characterized by multiploidy (Fig. 1E, 20 h in c–f). These data suggest that if mitotic DNA damage can be recovered from, multiploid cells will divide normally, but serious damage causes cellular defects.

Accumulation of cells in G1 phase with 4 N-DNA contents within 8 h of release was not changed by treatment with hydroxyurea in comparison with that without treatment. However, multiploid cells containing 8 N-DNA content totally disappeared after release for 20 h with hydroxyurea treatment (Fig. 2B, 20 h in d–f). Bromodeoxyuridine (BrdU) incorporation was starting in 5-hour releasing time point in cells with mitotic DNA damage (Fig. 2, b in C and BrdU-FITC in b, D). After 20 h-incubation, nuclear BrdU were diffused into the whole cytoplasm under the damage condition (Fig. 2D, 20 h in b). These data suggested that suggesting that mitotic cells containing DNA damage do not progress into cytokinesis and enter new interphase with 4 N-DNA content, whereas re-replication of 4 N-DNA occurs once during continuous culture without further cell division.

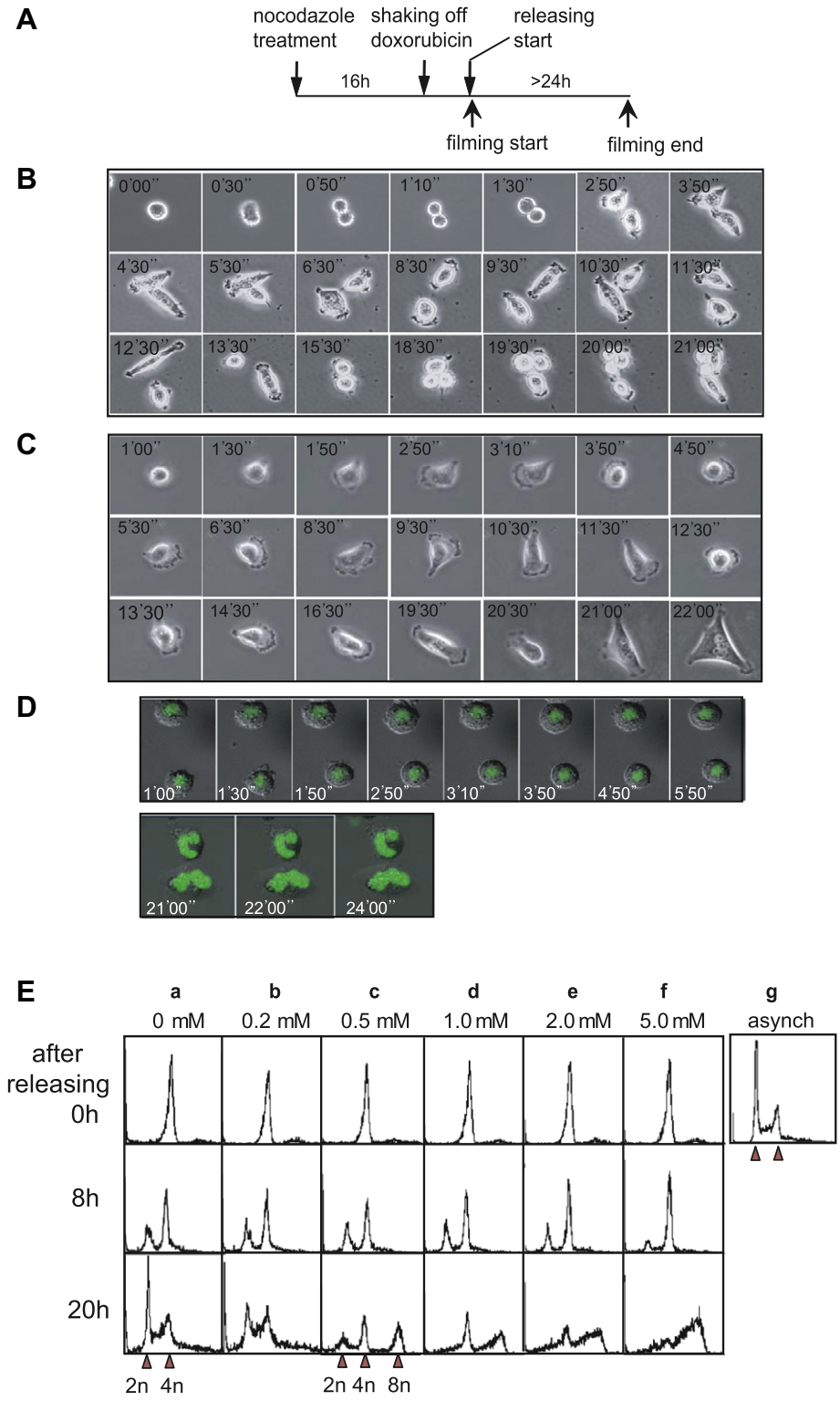

Fig. 1. Mitotic cell with DNA damage did not undergo cytokinesis. (A) HeLa cells were treated with nocodazole for 16 h to synchronize in prometaphase, and following shaking-off the mitotic cells, treated with doxorubicin for 1 h. Mitotic cells with DNA damage are released in fresh media for recovery, and were observed under time lapse microscopy. Mitotic cells without DNA damage progress into cytokinesis (B), but mitotic cells damaged by treatment with doxorubicin did not undergo cytokinesis and remained as single cell in prolonged culture until ~20 h (C). (D) HeLa cells expressing histone H2B-GFP were cultured under the previous condition. Mitotic cells with DNA damage are released in fresh media for recovery, and were observed under time lapse microscopy. (E) Mitotic cell with DNA damage was accumulated in 8 N-DNA content (multiploidy) during prolonged culture for 24 h. Arrowheads indicated 8 N-DNA index. (a) Untreated with doxorubicin; (b) treated with 0.2 μM of doxorubicin; (c) treated with 0.5 μM of doxorubicin; (d) treated with 1.0 μM of doxorubicin; (e) treated with 2.0 μM of doxorubicin; (f) treated with 5.0 μM of doxorubicin; (g) DNA contents of unsynchronous cells.

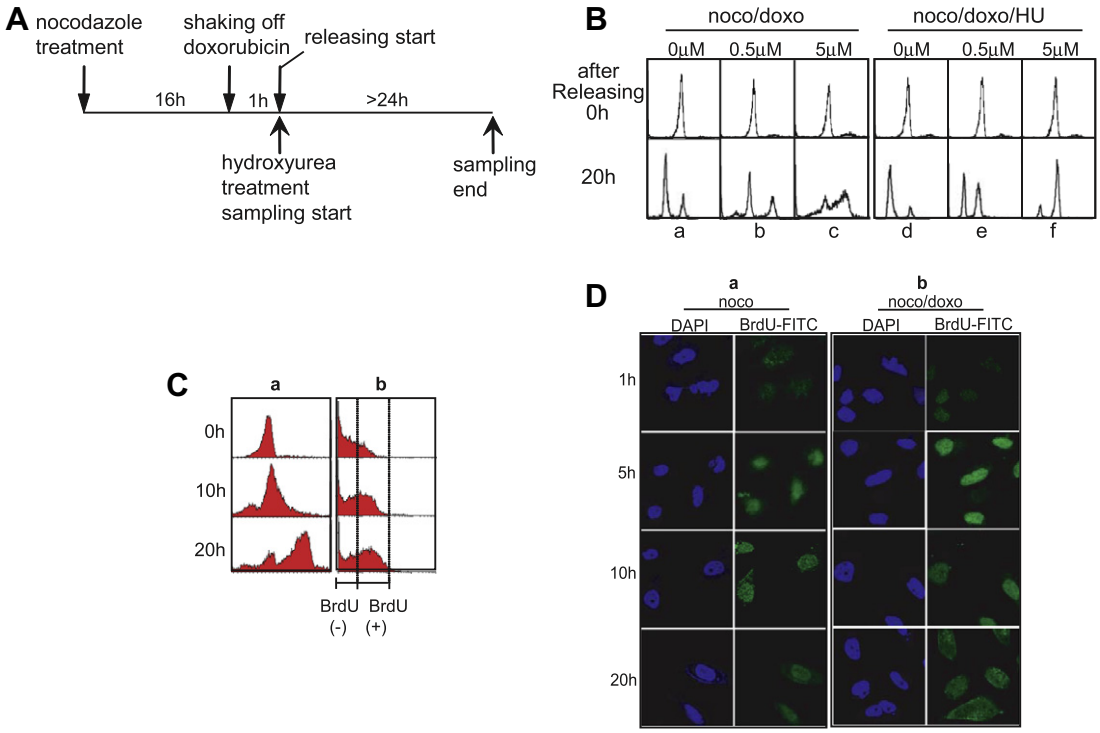

Fig. 2. Multiploidy was caused by re-replication of 4 N-DNA in mitotic cell with DNA damage. (A) Flowchart for treatment of hydroxyurea in mitotic cells with DNA damage. (B) Mitotic cells treated with doxorubicin accumulated in multiploid state during releasing for 20 h (a–c). Multiploidy cells were not detected by hydroxyurea treatment during releasing (d–f). (a) untreated with doxorubicin; (b) treated with 0.5 μM of doxorubicin; (c) treated with 5.0 μM of doxorubicin; (d) untreated with hydroxyurea; (e) treated with hydroxyurea and 0.5 μM of doxorubicin; (f) treated with hydroxyurea and 5.0 μM of doxorubicin. (C) Bromodeoxyuridine (BrdU) incorporation was increased during releasing for indicated time points in fresh media (b). DNA contents of cells were presented in (a). (D) Nuclear concentration of BrdU during releasing for indicated time points. Mitotic cells without DNA damage (a) or with DNA damage (b) were cultured for indicated time and fixed for confocal microscopic analysis.

3.2. Mitotic DNA damage causes final cell death through apoptosis in prolonged culture

After 24 ~ 72 h of releasing, most of cells with 8 N-DNA content dramatically accumulated in sub-G_0 phase (Fig. 3A, 48 h and 72 h in b). When we performed Western Blot analysis to detect the activation of major apoptotic factors during prolonged culture, the active cleavage of PARP-1 and caspase-3 were observed in cells (Fig. 3B, lanes 3 & 4 in upper & middle panels). Indeed, upon treatment with a caspase inhibitor, Z-VAD-FMK (Fig. 3C), the active cleavages of PARP and caspase-3 were not detected even 72 h after release (Fig. 3D, lane 3 in upper & middle panels). This result indicates that final cell death in the mitotic DNA damage response in cells follows the caspase-dependent apoptotic pathway. Under this condition, induction of multiploidy by re-replication might not be an essential factor for apoptotic cell death (data not shown).

3.3. Induction of multiploidy and long-term viability in mitotic DNA damage response is dependent on the ATM/Chk1 pathway

Previously, we showed that mitotic DNA damage induced dephosphorylation of Plk1 activated through the ATM-Chk1-PP2A pathway, and cells accumulated in interphase with 4N DNA content [16,17]. Dephosphorylation and inactivation of mitotic Plk1 might be important for the mitotic DNA damage response as a downstream effecter in the ATM/Chk1 pathway [16,17]. After 8 h of release, mitotic cells with DNA damage stayed in 4 N-DNA stage as expected (Fig. 4B, 8 h in a). When Chk1 was depleted in mitotic cells with DNA damage, the accumulation of neither 4 N-DNA cells nor multiploidy was not detected (Fig. 4B, 8 h and 20 h in b). Moreover, depletion of Chk1 induced cell death quickly in the mitotic DNA damage response within 12 h of incubation (Fig. 4B, 12 h and 20 h in b, asterisk). When ATM was depleted in mitotic cells with DNA damage, cell death was increased within 12 h of incubation and multiploidy was not detected (Fig. 4B, c). After transfection of shRNA construct of ATM and Chk1 for 24 h (Fig. 4A), expression of endogenous proteins were decreased (Fig. 4C, lanes 2 & 3 in a & b). These data suggest that accumulation of G1 cells containing 4 N-DNA content occur in ATM/Chk1-dependent manner [16,17], and that long-term survival of multiploid cells for 2–3 days might be induced by ATM/Chk1 pathway in the mitotic DNA damage.

4. Discussion

DNA damage during cell division cycle activates a checkpoint pathway involving damage to two sensing kinases, ATM/ATR, and their downstream protein kinases, such that cells stop growing until the damage is repaired. If the repair process is successful, the checkpoint response is deactivated, and cells enter a state of recovery. Therefore, activation of the DNA damage response results in transient arrest in cell division, repair, and final apoptosis for clearance of unrepaired cells. To investigate whether or not the mitotic DNA damage checkpoint follows this general response to DNA damage, mitotic cells with various levels of DNA damage were incubated in fresh media for recovery.

In previous reports, we mentioned a response to DNA damage during mitosis, not interphase, and showed that DNA damage induces accumulation of mitotic cells in interphase with 4 N-DNA content and not in mitosis within 2 ~ 3 h [17]. Under this condition, two major mitotic protein kinases, Cdk1 and Plk1, become inactivated by inhibitory phosphorylation and dephosphorylation, respectively, without large effects on cyclin B levels [16]. Consistently, time-lapse microscopy and FACS analysis in this report

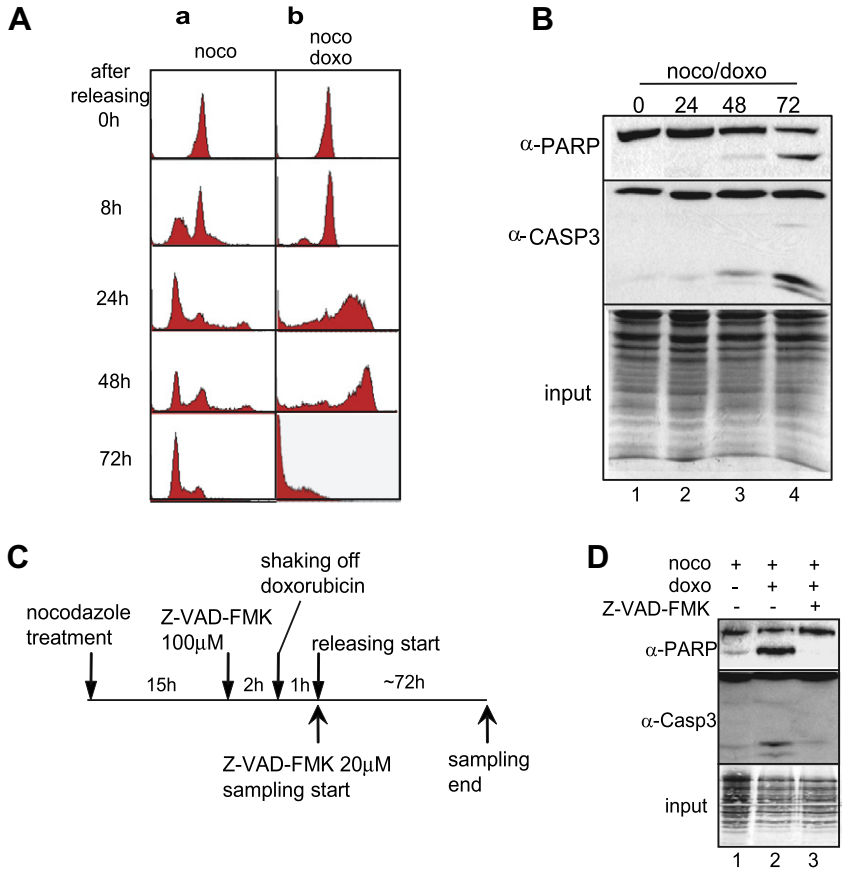

Fig. 3. Cell death of 8 N-DNA cells followed by a caspase-dependent apoptosis pathway. (A) Most of cells damaged were accumulated in sub-G0 phase after releasing for 72 h. Normal mitotic cells were accumulated in G1 phase in the same time after washing. (a) Mitotic cells without damage; (b) cells with mitotic DNA damage; (B) Western analysis for detection of active fragments of PARP (α-*PARP*) and caspase-3 (α-*Casp3*). At indicated time point of releasing, cells were harvested, and proteins were separated on SDS–PAGE. The active fragments were indicated as arrowheads. (C) Mitotic cell with DNA damage was treated with Z-VAD-FMK before DNA damage shock, washed, and released into fresh media contained Z-VAD-FMK for 72 h. (D) Inhibition of the active cleavages of PARP (α-*PARP*) and caspase-3 (α-*Casp3*) by treatment with Z-VAD-FMK. (1) Cells released from mitosis for 72 h; (2) cells with mitotic DNA damage; (3) cells with mitotic DNA damage and Z-VAD-FMK treatment.

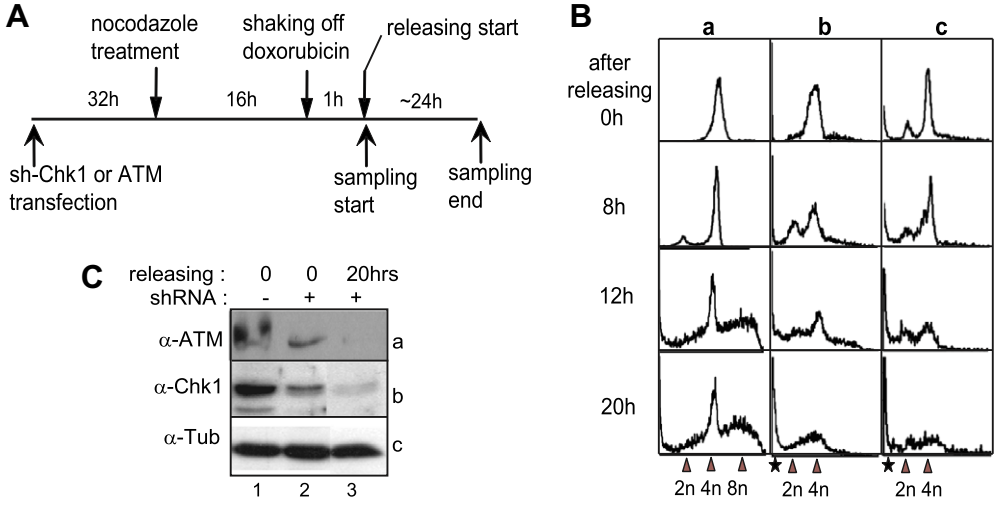

Fig. 4. Induction of multiploidy in mitotic DNA damage response is dependent on ATM/Chk1 pathway. (A) Scheme for ATM or Chk1 depletion and mitotic DNA damage. (B) Effect of ATM or Chk1 depletion on mitotic DNA damage response. Cells depleted ATM or Chk1 defected within 24 h, and did not show multiploidy. (a) Mitotic cells with DNA damage; (b) mitotic cells with DNA damage and depletion of Chk1; (c) mitotic cells with DNA damage and depletion of ATM. (C) Western Blot of ATM (a) and Chk1 (b) depleted by treatment of sh-RNAs. (1) No shRNA; (2) knock down with shRNAs for 32 h; (3) knock down with shRNAs for 52 h. α-Tubulin signal was used as a control for quantification of total proteins (c).

showed that mitotic cells with DNA damage did not undergo cytokinesis and entered G1 phase with 4 N-DNA content upon short-term release (Fig. 1C, E), and that accumulation of 4N-G1 within 8 h was dependent on the level of DNA damage (Fig. 1E). These results indicate that according to inactivation of major mitotic protein kinases through the ATM/Chk1/PP2A pathway, cells cannot

progress through the remaining steps of mitosis, including cytokinesis. Under these conditions, although mitotic DNA damage induced to inhibit cytokinesis, cells could enter interphase with 4 N-DNA without chromosome segregation in most of the damaged cells, and one round of DNA replication occurred.

The next issue in the mitotic DNA damage response is the emergence of multiploidy (Fig. 1E, 20 h in c–f). Treatment with hydroxyurea and BrdU incorporation indicated that 4N-G1 cells entered re-replication and contained 8 N-DNA contents (Fig. 2). DNA replication seemed to start within 10 h after the start of incubation (Fig. 2C, D, 10 h in b). Interestingly, after 20 h of release in fresh media for recovery, a multi-nuclei phenotype was observed in time-lapse microscopic analysis, indicating that nuclear division halted by the DNA damage response began repeatedly without further cell division, and that induction of multiploidy by re-replication and nuclear division are features of adaptation in the mitotic DNA damage checkpoint. In our reports, accumulation in 4N-G1 phase of mitotic cells with DNA damage during short incubation for 6–8 h was found in most of cell lines [16]. However, formation of multiploidy during prolonged culture was not general tendency (Supplementary data-1). According to cancer types, 4N-G1 cells were not arrested, processed DNA replication, and became 8 N-DNA. The molecular mechanism of cell type-specific induction of multiploidy in mitotic DNA damage response will be studied in future. Previously, cells during prolonged treatment with nocodazole can adapt and exit, indicating that the adapted cells do not undergo cell division, enter 4N-G1, and are characterized by multi-nuclei [21]. Similarly, cells that cannot satisfy the spindle assembly checkpoint are delayed in mitosis. However, this delay escapes and enters G1 phase as tetraploid cells in the presence of an active spindle assembly checkpoint. One of the ways to adapt is inhibition of Cdk1 activity without affecting cyclin B1 levels in yeast and Drosophila [18,22]. Since cells bypassed anaphase in our experiments, the anaphase-promoting complex might not have been activated. Indeed, the levels of cyclin B1 as well as Plk1, which are major substrates of the anaphase-promoting complex, remained high. Instead, Cdk1 and Plk1 were inactivated by inhibitory phosphorylation and dephosphorylation, respectively, in similar phenomena in yeast and Drosophila as previously mentioned.

Although nocodazole treatment for synchronizing and gathering mitotic cells had no side-effects on normal cell cycle progression (Fig. 1E, a), the mitotic DNA damage response proposed here showed a similar phenotype with that of the delayed mitotic response as mentioned in a previous report [23].

A powerful response to DNA damage checkpoint promotes repair of damaged cells and finally clears cells that are beyond repair through apoptosis. Prolonged mitotic arrest can induce apoptosis in mitosis. After 3 days or longer of incubation for recovery, mitotic cells with DNA damage finally died via caspase-dependent apoptosis (Fig. 3A, B), and cleavage of caspase-3 for activation did not occur in response to treatment with Z-VAD-fmk (Fig. 3D). Moreover, the doxorubicin concentration for DNA damage was important in defining the fate of this mitotic DNA damage response. In response to lower doxorubicin concentration (less than 0.5 μM), most mitotic cells escaped multiploidy, and went through to cell division to have normal DNA contents within 24 h (Fig. 1E, b). In contrast, when exposed to higher doxorubicin concentrations, cells remarkably accumulated multiploidy. Moreover, cells treated with a high dosage of DNA damage had great sensitivity to ATM and Chk1 deficiency (Fig. 1E, c–f and Fig. 4B). Based on this situation, there is no doubt that the molecular phenotype with regard to inactivation of two major mitotic protein kinases, Plk1 and Cdk1, is an important factor. In summary, the prometaphasic cells with DNA damage bypassed later mitosis and cytokinesis and entered G1 phase with 4 N-DNA content. After a prolonged delay, DNA replication occurred to be 8N-multiploidy (Supplementary data-2). Then, due to remnants of Plk1 (even it is inactivated) and cyclin B1, nuclear division was induced without cytokinesis [24]. If the damage cannot be recovered from, cells were removed by apoptosis. Again, as in all checkpoints, mitotic the DNA damage checkpoint proposed in this report follows a conserved process. First, when mitotic DNA damage occurs, cell cycle transient is delayed. Second, this mitotic DNA damage checkpoint is activated specifically before starting anaphase. Third, mitotic cell with DNA damaged have to adaptation slippage and progress to G1 phase with 4 N-DNA contents without cytokinesis, and then adaptation in multiploidy. If DNA damage repair is complete, cells proceed with their cell cycle program, but when DNA damage repair is not complete, cells are removed by apoptosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.06.023.

References

[1] B.B. Zhou, S.J. Elledge, The DNA damage response: putting checkpoints in perspective, Nature 408 (2000) 433–439.
[2] J.C. Harrison, J.E. Haber, Surviving the breakup: the DNA damage checkpoint, Annu. Rev. Genet. 40 (2006) 209–235.
[3] J.H. Houtgraaf, J. Versmissen, W.J. van der Giessen, A concise review of DNA damage checkpoints and repair in mammalian cells, Cardiovasc. Revasc. Med. 7 (2006) 165–172.
[4] J. Bartek, J. Lukas, DNA damage checkpoints: from initiation to recovery or adaptation, Curr. Opin. Cell Biol. 19 (2007) 238–245.
[5] K. Suzuki, S. Kodama, M. Watanabe, Recruitment of ATM protein to double strand DNA irradiated with ionizing radiation, J. Biol. Chem. 274 (1999) 25571–25575.
[6] Z. Guo, A. Kumagai, S.X. Wang, W.G. Dunphy, Requirement for Atr in phosphorylation of Chk1 and cell cycle regulation in response to DNA replication blocks and UV-damaged DNA in Xenopus egg extracts, Genes Dev. 14 (2000) 2745–2756.
[7] K. Unsal-Kacmaz, A.M. Makhov, J.D. Griffith, A. Sancar, Preferential binding of ATR protein to UV-damaged DNA, Proc. Natl. Acad. Sci. U. S. A 99 (2002) 6673–6678.
[8] R. Boutros, C. Dozier, B. Ducommun, The when and wheres of CDC25 phosphatases, Curr. Opin. Cell Biol. 18 (2006) 185–191.
[9] H. Niida, M. Nakanishi, DNA damage checkpoints in mammals, Mutagenesis 21 (2006) 3–9.
[10] C. Karlsson-Rosenthal, J.B. Millar, Cdc25: mechanisms of checkpoint inhibition and recovery, Trends Cell Biol. 16 (2006) 285–292.
[11] A.L. Corwin, T.C. Dai, D.D. Duc, P.I. Suu, N.T. Van, L.D. Ha, M. Janick, L. Kanti, A. Sie, R. Soderquist, R. Graham, S.F. Wignall, K.C. Hyams, Acute viral hepatitis in Hanoi, Viet Nam, Trans. R. Soc. Trop. Med. Hyg. 90 (1996) 647–648.
[12] H. Ohkura, I.M. Hagan, D.M. Glover, The conserved *Schizosaccharomyces pombe* kinase plo1, required to form a bipolar spindle, the actin ring, and septum, can drive septum formation in G1 and G2 cells, Genes Dev. 9 (1995) 1059–1073.
[13] S. Kotani, S. Tugendreich, M. Fujii, P.M. Jorgensen, N. Watanabe, C. Hoog, P. Hieter, K. Todokoro, PKA and MPF-activated polo-like kinase regulate anaphase-promoting complex activity and mitosis progression, Mol. Cell 1 (1998) 371–380.
[14] V.A. Smits, R. Klompmaker, L. Arnaud, G. Rijksen, E.A. Nigg, R.H. Medema, Polo-like kinase-1 is a target of the DNA damage checkpoint, Nat. Cell Biol. 2 (2000) 672–676.
[15] M.A. van Vugt, V.A. Smits, R. Klompmaker, R.H. Medema, Inhibition of polo-ke kinase-1 by DNA damage occurs in an ATM- or ATR-dependent fashion, J. Biol. Chem. 276 (2001) 41656–41660.
[16] H.J. Lee, H.I. Hwang, Y.J. Jang, Mitotic DNA damage response: polo-like kinase-1 is dephosphorylated through ATM-Chk1 pathway, Cell Cycle 9 (2010) 2389–2398.
[17] Y.J. Jang, J.H. Ji, Y.C. Choi, C.J. Ryu, S.Y. Ko, Regulation of polo-like kinase 1 by DNA damage in mitosis. Inhibition of mitotic PLK-1 by protein phosphatase 2A, J. Biol. Chem. 282 (2007) 2473–2482.

[18] S.J. Suijkerbuijk, G.J. Kops, Preventing aneuploidy: the contribution of mitotic checkpoint proteins, Biochim. Biophys. Acta 1786 (2008) 24–31.
[19] C. Mantel, Y. Guo, M.R. Lee, M.K. Han, S. Rhorabough, K.S. Kim, H.E. Broxmeyer, Cells enter a unique intermediate 4N stage, not 4N–G1, after aborted mitosis, Cell Cycle 7 (2008) 484–492.
[20] M. Castedo, J.L. Perfettini, T. Roumier, K. Andreau, R. Medema, G. Kroemer, Cell death by mitotic catastrophe: a molecular definition, Oncogene 23 (2004) 2825–2837.
[21] R. Panvichian, K. Orth, M.L. Day, K.C. Day, M.J. Pilat, K.J. Pienta, Paclitaxel-associated multimininucleation is permitted by the inhibition of caspase activation: a potential early step in drug resistance, Cancer Res. 58 (1998) 4667–4672.
[22] E. Foley, F. Sprenger, The cyclin-dependent kinase inhibitor Roughex is involved in mitotic exit in Drosophila, Curr. Biol. 11 (2001) 151–160.
[23] C.L. Rieder, H. Maiato, Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint, Dev. Cell 7 (2004) 637–651.
[24] X. Huang, T. Tran, L. Zhang, R. Hatcher, P. Zhang, DNA damage-induced mitotic catastrophe is mediated by the Chk1-dependent mitotic exit DNA damage checkpoint, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 1065–1070.