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Requirement of a Functional Spindle Checkpoint for Arsenite-Induced Apoptosis

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ABSTRACT

To understand the potential influence of spindle checkpoint function in response to arsenic trioxide (ATO)-induced apoptosis observed in cancer cell lines, we examined the correlation between activation of the spindle checkpoint and susceptibility to ATO-induced apoptosis in 10 cancer cell lines lacking functional p53. The ability to functionally activate the spindle checkpoint in each cancer cell line was assessed by the induction of mitotic arrest after Taxol treatment. Bromodeoxyuridine (BrdU) pulse-chase analysis of Taxol-treated cell lines with low mitotic arrest showed that they were not arrested at mitosis but divided abnormally, confirming that spindle checkpoint activation was impaired in these cell lines. Our results demonstrate that apoptosis was significantly induced by ATO in cancer cell lines with functional activation of the spindle checkpoint and substantial induction of mitotic arrest. Cell lines with negligible mitotic arrest exhibited little ATO-induced apoptosis. However, no such correlation was observed following treatment of cells with camptothecin, a topoisomerase I inhibitor. Furthermore, attenuation of the spindle checkpoint function by small interfering RNA–mediated silencing of BubR1 and Mad2 in cancer cells that were susceptible to ATO-induced mitotic arrest and apoptosis greatly reduced the induction of mitotic arrest and apoptosis by ATO and increased the formation of micronuclei or multinuclei in survived cells. The marked correlation between ATO-induced mitotic arrest and apoptosis indicates that the induction of apoptosis by ATO was highly dependent on the functional activation of the spindle checkpoint in cancer cells lacking normal p53 function. J. Cell. Biochem. 105: 678–687, 2008.

KEY WORDS: ARSENIC TRIOXIDE; TAXOL; SPINDLE CHECKPOINT; MITOTIC ARREST; APOPTOSIS

Arsenic trioxide (ATO) has proved effective in the treatment of patients with newly diagnosed and relapsed acute promyelocytic leukemia (APL) [Soignet et al., 1998]. The therapeutic effects of ATO are dose-dependent and mainly result from the specific degradation of the leukemogenic protein encoded by the retinoic acid receptor α (*RARα*)/promyelocytic leukemia (*PML*) fusion gene and the resulting induction of non-terminal cytodifferentiation at lower concentrations (≤0.5 μM) and induction of apoptosis at higher concentrations (0.5–2 μM) [Chen et al., 1997]. However, several studies have demonstrated that PML-RARα-negative cell lines and cells from PML knockout mice are also sensitive to ATO [Wang et al., 1998; Zhu et al., 1999]. In addition, ATO has been shown to induce apoptosis in several solid tumor cell lines [Zhang et al., 1999; Ora et al., 2000; Ling et al., 2002; Hyun Park et al., 2003]. These studies suggest that apoptosis induction could be an important therapeutic effect of ATO on cancers in addition to APL.

Arsenite-induced apoptosis has been reported to be associated with activation of caspase activity [Chen et al., 1996; Akao et al., 1998], enhanced generation of reactive oxygen species (ROS) [Wang et al., 1996], opening of mitochondrial permeability transition pores [Zheng et al., 2003], inhibition of NF-κB [Mathas et al., 2003], and sustained activation of c-jun-terminal kinase [Kajiguchi et al., 2003]. However, the mechanism by which arsenite triggers apoptosis remains controversial. The activation of particular cellular processes by ATO appears to be dependent on drug concentration, cell types, and cellular environment. Many reports indicate that arsenite might induce G1 arrest in a p53-dependent manner [Yih and Lee, 2000; States et al., 2002; Liu et al., 2003]. Loss of p53 function sensitizes cells to arsenite-induced mitotic arrest and induces apoptosis in mitotically arrested cells [Taylor et al., 2006]. In addition, arsenic compounds can disrupt mitosis by interfering with tubulin polymerization and disrupting mitotic spindles [Huang and Lee, 1998; Li and Broome, 1999; Ling et al., 2002], inducing mitotic arrest, and con-sequently triggering mitotic arrest–mediated apoptosis in a variety of cells carrying p53 mutations [Huang et al., 2000; Ling et al., 2002; States et al., 2002; Cai et al., 2003; Liu et al., 2003; Yih et al., 2005]. These reports demonstrate a tight link between arsenite-induced apoptosis and mitotic arrest in cancer cells without functional p53.

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We have previously demonstrated that arsenite in a clinical achievable concentration can alter mitosis progression and induce mitotic arrest [Yih et al., 1997; Yih and Lee, 2003] and that arsenite can induce mitotic abnormalities and mitosis-mediated apoptosis in a spindle checkpoint–dependent manner [Yih et al., 2006]. Mitosis-mediated apoptosis, a type of cell death occurs during or after aberrant mitosis, is now recognized as an important mechanism underlying the therapeutic effects of several microtubule-disrupting drugs. Functional activation of the spindle checkpoint is required for apoptosis induction in response to microtubule-disrupting agents [Masuda et al., 2003; Sudo et al., 2004; Vogel et al., 2005]. Spindle checkpoint defects in human tumors have been correlated with resistance to the microtubule-stabilizing chemotherapeutic drug Taxol [Jordan and Wilson, 2004]. To characterize the role of spindle checkpoint function in cellular susceptibility to ATO, 10 cancer cell lines comprising bladder, cervical, lung, ovarian, and colon cancers and all lacking normal p53 function, were adopted to examine the correlation between the spindle checkpoint activity and sensitivity to ATO-induced apoptosis.

MATERIALS AND METHODS

CELL CULTURE

The tumorigenic cell line CGL-4 [Stanbridge et al., 1981], derived from a hybrid (ESH5) of the HeLa variant, D98/AH2, and a normal human fibroblast strain, GM77, were kindly provided by Dr. E. J. Stanbridge (University of California, Irvine). BFTC905 [Tzeng et al., 1996] and NTU-B1 [Yu et al., 1992] cells were obtained from the Biosource Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan). C-33A, C4-II, H-1299, H-322, OVCAR-3, SW480, and T-24 cells were obtained from the American Type Culture Collection (Manassas, VA). BFTC905, NTUB1, and T24 are human bladder urothelial carcinoma cell lines; C-33A and C4-II are cervical carcinoma cell lines; H1299 and H322 are lung cancer cell lines; OVCAR-3 is an ovarian cancer cell line; and SW480 is a colorectal adenocarcinoma cell line. The cells were routinely maintained in Dulbecco's modified Eagle medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen), 0.37% sodium bicarbonate, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified incubator in air and 10% CO_2. The cells were passaged twice per week.

DRUG TREATMENT

Logarithmically growing cells were left untreated or treated with 1, 2, or 4 μM ATO (Sigma, Saint Louis, MO), 5, 10, or 20 nM Taxol (Calbiochem, Merck Biosciences, San Diego, CA), or 10, 20, or 40 ng/ml camptothecin (Sigma). The cells were treated for 24 h for cell cycle distribution analysis and 48 h for apoptosis analysis. The stock solution of ATO (10 mM) was prepared in 0.1 N NaOH, and the stock solutions of Taxol (0.1 mM) and camptothecin (0.1 mg/ml) were prepared in dimethyl sulfoxide and stored at −20°C in aliquots.

ANALYSIS OF CELL CYCLE DISTRIBUTION AND SPINDLE CHECKPOINT ACTIVATION

Cell cycle progression was monitored using DNA flow cytometry. DNA was stained with propidium iodide (PI), and mitotic cells were quantified by measuring the expression of a mitosis-specific marker, phospho-histone H3 [Yih et al., 2006]. After treatment with each drug for 24 h, the cells were trypsinized, washed once with phosphate buffered saline (PBS), pH 7.4, fixed with ice-cold 70% ethanol for 16 h, then immunostained with a mouse anti-phospho-histone H3 (serine 10) antibody (#9706, Cell Signaling Technology, Beverly, MA), followed by a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). The cells were then stained with 4 μg/ml PI in PBS containing 1% Triton X-100 and 0.1 mg/ml of RNase A. Phospho-histone H3 levels and the DNA content of individual cells were analyzed using a fluorescence-activated cell sorter (Epics® XL/MCL, Beckman Coulter, Fullerton, CA), and the cell cycle distribution of the cells was determined using a computer program provided by Beckman Coulter, as described previously [Yih et al., 2005]. Because induction of mitotic arrest in response to microtubule-disrupting drugs can imply activation of the spindle checkpoint [Cahill et al., 1998; Takahashi et al., 1999; Tighe et al., 2001; Wang et al., 2002], impaired activation of the spindle checkpoint was defined as a mitotic index lower than 20% after 10–20 nM Taxol treatment for 24 h [Masuda et al., 2003].

DETECTION OF APOPTOSIS

The number of apoptotic cells was determined using an Annexin V-FITC apoptosis detection kit (Oncogene, Boston, MA) as described previously [Yih et al., 2005]. After treatment with each drug for 48 h, the cells were collected, washed once with PBS, and resuspended in 100 μl of binding buffer containing 5 μl of a 200 μg/ml solution of FITC-conjugated Annexin V and 5 μl of a 30 μg/ml solution of PI. After a 10-min incubation at room temperature, FITC binding to individual cells was analyzed using a fluorescence-activated cell sorter. The percentage of apoptotic cells (FITC-positive) in 10,000 cells was calculated in each experiment.

BROMODEOXYURIDINE (BrdU) INCORPORATION AND ANALYSIS

Logarithmically growing cells were treated with 20 μM BrdU for 30 min. BrdU was then removed and the cells were further incubated in drug-free medium or medium containing Taxol or ATO. At various time points, cells were harvested for analysis of BrdU incorporation and DNA content. In brief, total cells were collected and fixed in cold 70% ethanol. After treatment with 2 N HCl to nick the DNA, 0.1 M $Na_2B_4O_7$ was added to neutralize the reaction. The incorporated BrdU was reacted with FITC-conjugated anti-BrdU (Roche Applied Science, Mannheim, Germany), and cellular DNA was stained with 4 μg/ml PI in PBS containing 1% Triton X-100 and 0.1 mg/ml RNase A. The fluorescence intensities of PI and FITC of individual cells were analyzed with a fluorescence activated cell sorter as previously described [Yih and Lee, 2000]. The BrdU level in each cell cycle phase was determined using a computer program provided by Beckman Coulter.

DETECTION OF NUCLEAR FRAGMENTATION, MICRONUCLEATION, AND MULTI-NUCLEATION

After treatments, cells were either in vivo labeled with 0.5 μM Hoechst 33342 (Invitrogen) for 15 min and visualized under a inverted fluorescence microscope (Zeiss Axiovert 200M,

Oberkochen, Germany) or fixed in 90% methanol, stained with 0.1 μg/ml of 4,6-diamino-2-phenyl-indole (DAPI, Sigma), and visualized under a fluorescence microscope (Zeiss Axioskop 2). To calculate the percentage of cells with micronuclei or multinuclei, three independent experiments were performed and at least 500 cells were analyzed in each experiment.

RNA INTERFERENCE

RNA interference was carried out as described previously [Elbashir et al., 2001]. The small interfering RNA (siRNA) was synthesized by Proligo Singapore Pte Ltd. (Singapore). Cells (1×10^5 per 60-mm dish) were plated 1-day before transfection; siRNA was transfected into the cells using Oligofectamine (Invitrogen) at a final concentration of 50 nM. At 24 h post-transfection, the medium was replaced with fresh medium and the cells treated with Taxol or ATO. A double-stranded RNA targeting luciferase (5′-cguacgcg-gaauacuucgadTdT-3′) was used as a control. The sequences targeted by the BubR1 and mitotic arrest deficient two (Mad2) siRNAs were 5′-cacttatggtactgtatat-3′ and 5′-ctactacaatccacaaagt-3′, respectively.

IMMUNOBLOTS

Cellular expression of BubR1 and Mad2 was examined by immunoblot analysis. Briefly, all cells were collected, washed twice with ice-cold PBS, and boiled in SDS–PAGE sample buffer [Laemmli, 1970]. Samples containing equal amounts of cellular proteins (20–50 μg) were resolved by 8% SDS–PAGE, transferred onto polyvinylidene difluoride membranes (Amersham Biosciences, Piscataway, NJ), and incubated overnight at 4°C with the primary antibodies diluted in PBST, then for 1 h at room temperature with the appropriate horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch Laboratories) diluted in PBST. Following extensive washes with PBST, bound antibody was visualized by chemiluminescence using SuperSignal West Pico chemiluminescence reagent (Pierce, Rockford, IL). β-Actin was used as a loading control. The results shown are representative of at least two independent experiments. Goat anti-Mad2 antibody was purchased from Santa Cruz Biotechnology and mouse anti-BubR1 antibody and mouse anti-β-actin from Chemicon International (Temecula, CA). Protein concentrations were determined by Bradford [1976].

RESULTS

DIFFERENTIAL SENSITIVITY TO ATO-INDUCED MITOTIC ARREST IN CANCER CELL LINES LACKING p53 FUNCTION

Ten cancer cell lines, all lacking p53 function, were treated with 1–4 μM ATO for 24 h, respectively and the induction of mitotic arrest in each cell line was measured. The results showed that treatment of 1–4 μM ATO significantly increased the mitotic index to 20–40% in BFTC905, CGL-4, NTUB1, and OVCAR-3 cells (Fig. 1A). However, the mitotic index of other cancer cell lines, including C-33A, C4-II, H-1299, H-322, SW-480, and T24 cells, was less than 20% after ATO treatment (Fig. 1A). Increasing the ATO concentration to 10 μM did not induce a further increase in mitotic index in these cell lines (data not shown). These results indicated that cancer cell lines lacking p53 function have different sensitivity to ATO-induced mitotic arrest.

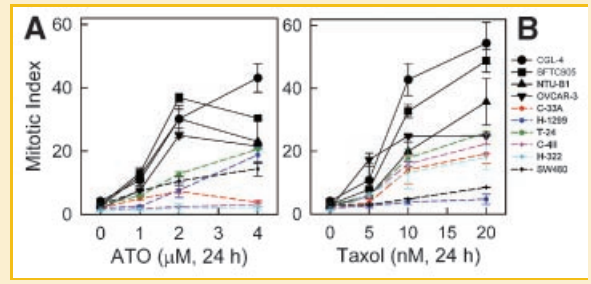

Fig. 1. Induction of mitotic arrest by ATO or Taxol in 10 cancer cell lines lacking p53 function. Cells were treated with 1–4 μM ATO **(A)** or 5–20 nM Taxol **(B)** for 24 h and harvested for analysis of cell cycle distribution. ATO- or Taxol-induced mitotic arrest was analyzed by staining cells with an antibody against phospho-histone H3 to label mitotic cells and with PI to label DNA. The results are the mean ± SD of 3–4 independent experiments.

Since induction of mitotic arrest implies functional activation of the spindle checkpoint in response to microtubule-disrupting drugs [Cahill et al., 1998; Takahashi et al., 1999; Tighe et al., 2001; Wang et al., 2002], we then hypothesize that no significant induction of mitotic arrest by ATO in cancer cell lines lacking p53 function might be due to impaired activation of the spindle checkpoint in these cells. Taxol was therefore adopted to examine whether the spindle checkpoint could be functionally activated in cancer cell lines lacking p53 function. The results show that cell lines that could be significantly arrested at mitosis by ATO, including BFTC905, CGL-4, NTUB1, and OVCAR-3 cells, could also be greatly arrested at mitosis by Taxol (Fig. 1B). Accordingly, the cell lines that could not be efficiently arrested at mitosis by ATO, namely C-33A, C4-II, H-1299, H-322, SW-480, and T-24 cells, also could not be prominently arrested at mitosis by Taxol. Impaired activation of the spindle checkpoint has been defined as a mitotic index lower than 20% after 10–20 nM Taxol treatment for 24 h [Masuda et al., 2003]. Our results indicated that activation of the spindle checkpoint might be impaired in C-33A, C4-II, H-1299, H-322, SW-480, and T24 cells and hence no significant induction of mitotic arrest by ATO or Taxol in these cancer cell lines.

INDUCTION OF ABNORMAL CELL DIVISION IN CANCER CELL LINES THAT COULD NOT BE ARRESTED AT MITOSIS BY TAXOL

Among the cancer cell lines that could not be arrested at mitosis by ATO or Taxol in Figure 1, SW-480 [Cahill et al., 1998] and T-24 cells [Doherty et al., 2003] was demonstrated to have an impaired spindle checkpoint function in response to microtubule-disrupting drugs. To ascertain that there was no proper activation of the spindle checkpoint in other cancer cell lines used in this study, the cell cycle progression of C-33A and H-1299 cells was examined in more detail by BrdU pulse-chase analysis and compared with that of CGL-4 cells. After a 30-min pulse of BrdU, the BrdU-labeled CGL-4 (Fig. 2A, left panel), C-33A (Fig. 2B, left panel), and H-1299 cells (Fig. 2C, left panel) progressed to the G2/M stage within 6 h and divided and entered into the subsequent G1 stage after 18 h of chase. These BrdU-labeled cells kept cycling as they peaked at the G2/M stage after 24 h of chase and then at the G1 stage after 40 h of chase.

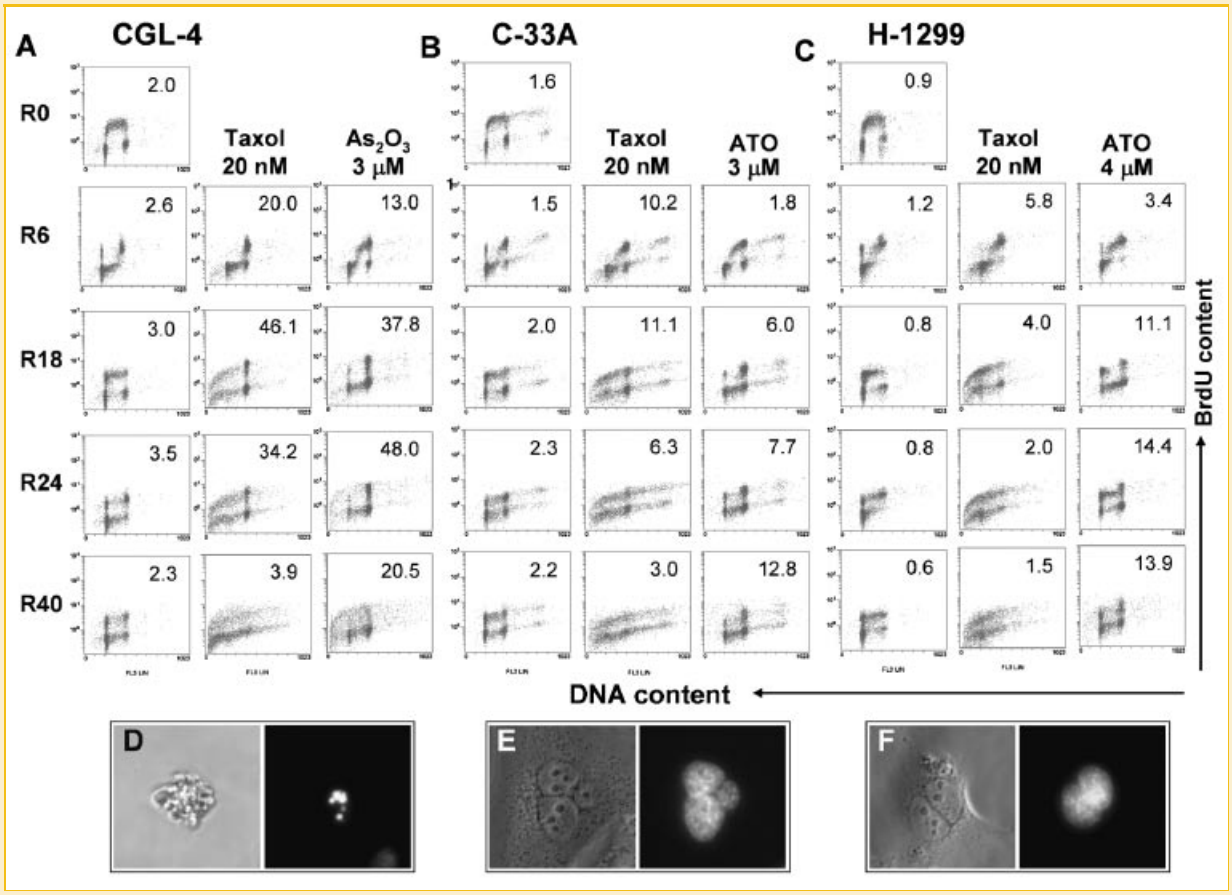

Fig. 2. Taxol induced abnormal cell division in C-33A and H1299 cells. CGL-4 (A), C-33A (B), or H-1299 (C) cells were pulse-labeled with 20 μM BrdU for 30 min (R0), chased in drug-free medium or medium containing 20 nM Taxol or 3–4 μM ATO for 6 (R6), 18 (R18), 24 (R24), or 40 h (R40). The BrdU-labeled cells in each treatment were monitored for the progression to each stage by staining with FITC-conjugated anti-BrdU. Nuclear DNA was counterstained with PI. The number in the upper right corner of each panel indicates the mitotic index. The results are a representative of two independent experiments. D–F: Morphology of Taxol-treated cells, an apoptotic CGL-4 (D), a mutltinucleated C-33A cell (E), and a binucleated H-1299 cell (F). The cells were treated with 20 nM Taxol for 40 h and then the nuclei were labeled with 0.5 μM Hoechst 33342 for 15 min and visualized under the inverted fluorescence microscope (Zeiss Axiovert 200M).

In the presence of 20 nM Taxol, the mitotic index of CGL-4 cells increased by 20% and 46.1% after 6 h and 18 h chase, respectively, compared to untreated cells (Fig. 2A, middle panel), indicating that Taxol-treated CGL-4 cells progressed into mitosis, where they became arrested. A large population of cells with very low DNA content appeared thereafter. Microscopic examination of these Taxol-treated CGL-4 cells revealed extensive cell blebbing (Fig. 2D, left) and nuclear fragmentation (Fig. 2D, right), both are characteristics of apoptosis. In contrast, the mitotic index of C-33A cells slightly increased by 10.2% and 11.1% after 6 and 18 h chase, respectively, in the presence of 20 nM Taxol (Fig. 2B, middle panel). These cells did not arrest at mitosis, as revealed by the subsequent decrease in mitotic index and a significant increase in cells with hypodiploid, diploid, or tetraploid DNA content at 18, 24, and 40 h (Fig. 2B, middle panel). Microscopic examination of these Taxol-treated C-33A cells revealed the formation of multiple nuclei instead of nuclear fragmentation in many cells (Fig. 2E). These results indicate that many of the Taxol-treated C-33A cells either kept progressing without cell division and became tetraploid or divided abnormally and became aneuploid. The BrdU pulse-labeled H-1299 cells progressed to the G2/M stage, and the mitotic index increased to 5.8% after 6 h chase in the presence of 20 nM Taxol (Fig. 2C, middle panel). Thereafter, the number of cells with hypodiploid or diploid DNA content increased dramatically after 18, 24 and 40 h of chase (Fig. 2B, middle panel). Microscopic examination of these Taxol-treated H-1299 cells also revealed the formation of multiple nuclei instead of nuclear fragmentation (Fig. 2F). These results indicated that many of the Taxol-treated H-1299 cells also divided abnormally and became aneuploid. Because previous studies have reported that Taxol treatment induced an aneuploid population in the absence of a proper mitotic block [Torres and Horwitz, 1998; Chen and Horwitz, 2002], our results indicate that the spindle checkpoint of C-33A and H-1299 cells was not appropriately activated in response to 20 nM Taxol and hence led to only a slight and transient mitotic arrest and subsequent abnormal cell division rather than apoptosis. These results suggest that the spindle checkpoint of these cancer cell lines was not functionally activated in response to Taxol treatment and imply that the spindle checkpoint and/or the regulatory machinery of the spindle checkpoint pathway were altered in these cancer cell lines.

To confirm that no efficient mitotic arrest was induced by ATO in cells having impaired spindle checkpoint activation, the cell cycle progression of ATO-treated C-33A and H-1299 was also examined with the BrdU-pulse chase analysis. In the presence of 3 μM ATO, the BrdU pulse-labeled CGL-4 cells progressed into and arrested at mitosis as the mitotic index was 37.8 and 48.0 after 18 and 24 h of chase. The mitotic index was then decreased and the cells with very low DNA content increased after 40 h of chase (Fig. 2A, right panel), indicating significant induction of mitotic arrest and apoptosis. However, with ATO treatment, the BrdU pulse-labeled C-33A cells progressed into the G2/M stage, although at a slower rate, as revealed by the fact that many of these cells remained at S phase after 6 h of chase and at the G2 stage after 18 h of chase in the presence of ATO (Fig. 2B, right panel). The non-BrdU-labeled cells also displayed retarded cell cycle progression in the presence of ATO. The mitotic index increased slightly to 6% after 18 h of chase. However, there was a significant increase in the number of G1 cells with BrdU signal after 18 h of chase in the presence of ATO, indicating that some of the BrdU-labeled cells could progress through mitosis, divide, and enter into the subsequent G1 stage. Thus, the cell cycle progression of C-33A cells was delayed but not arrested at mitosis in the presence of ATO. The ATO-treated H-1299 cells displayed a progression pattern similar to that of ATO-treated C-33A cells (Fig. 2C, right panel). These results confirm that no significant mitotic arrest was induced by ATO in C-33A or H-1299 cells and indicate that the spindle checkpoint was not activated by ATO in C-33A or H-1299 cells.

ATO-INDUCED APOPTOSIS CORRELATES STRONGLY WITH SPINDLE CHECKPOINT ACTIVATION

To understand whether activation of the spindle checkpoint and the resulting mitotic arrest were involved in ATO-induced apoptosis, induction of apoptosis was examined in cells treated with 1, 2, or 4 μM ATO or 5, 10, or 20 nM Taxol for 48 h. Apoptosis was significantly induced by Taxol in cell lines that could be substantially arrested at mitosis, whereas relatively less apoptosis was induced in cell lines with low induction of mitotic arrest (Fig. 3B). In addition, all the cancer cell lines with impaired spindle checkpoint activation in response to Taxol were relatively resistant to ATO-induced apoptosis (Fig. 3B). We further classified the 10 cell lines into two groups according to the level of mitotic arrest induced by treatment with 10 nM Taxol for 24 h. Cell lines with a mitotic index > 20% (Fig. 3D, +) were designated as having functional activation of the spindle checkpoint, and those with a mitotic index < 20% (Fig. 3D, -) were designated as having impaired activation of the spindle checkpoint [Masuda et al., 2003]. Figure 3D shows that the level of apoptosis induced by 2 μM ATO was significantly lower in cancer cell lines with impaired spindle checkpoint activation compared with cells having functional activation of the spindle checkpoint (the mean ± SD was

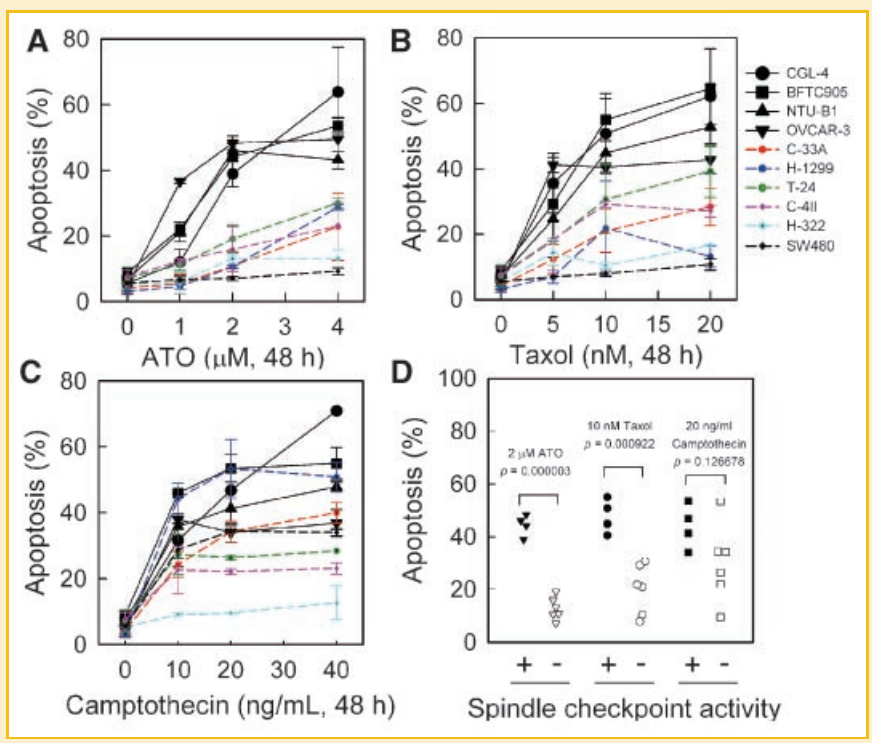

Fig. 3. ATO- or Taxol-induced apoptosis is highly dependent on induction of mitotic arrest in cancer cells. Cells were treated with 1–4 μM ATO (A), 5–20 nM Taxol (B), or 10–40 ng/ml camptothecin (C) for 48 h. Apoptosis was assessed by the cellular binding capacity of annexin V. The data represent the mean ± SD for 3–5 independent experiments. D: The 10 cancer cell lines were classified into two groups: +, mitotic index > 20% following treatment with 10 nM Taxol for 24 h; –, mitotic index < 20% following treatment with 10 nM Taxol for 24 h. Percent apoptosis induction was obtained from treatment of each cell line with 2 μM ATO, 10 nM Taxol, or 20 ng/ml camptothecin for 48 h. *P* was determined by the Student's *t*-test.

$12.76 \pm 4.27\%$ and $44.33 \pm 4.00\%$, respectively, $P < 0.001$ by Student's *t*-test). A significant difference in Taxol-induced apoptosis was also apparent between cells with or without functional spindle checkpoint activation (the mean $\pm$ SD was $20.16 \pm 9.38\%$ and $47.80 \pm 6.39\%$, respectively, $P < 0.001$ by Student's *t*-test). In contrast, the difference in the apoptosis induced by 20 ng/ml camptothecin, which inhibits topoisomerase I, results in DNA damage, and G2 arrest, was not significant between these two groups of cancer cell lines (Fig. 3C,D). Because induction of a significant mitotic arrest indicates a functional activation of the spindle checkpoint, our results implied that the induction of apoptosis by Taxol or ATO but not camptothecin was highly dependent on the functional activation of the spindle checkpoint.

ATTENUATION OF THE SPINDLE CHECKPOINT FUNCTION SIGNIFICANTLY REDUCES ATO-INDUCED MITOTIC ARREST AND APOPTOSIS

Activation of the spindle checkpoint in response to microtubule-targeting agents can be effectively abolished by depleting either one of the spindle checkpoint proteins BubR1 and Mad2 [Sudo et al., 2004]. The endogenous expression levels of BubR1 and Mad2 proteins in the 10 cancer cell lines used in this study were therefore examined. The results showed that, although the endogenous expression levels of BubR1 and Mad2 varied in each cancer cell line, both BubR1 and Mad2 were significantly accumulated after ATO treatment in cancer cell lines (CGL4, BFTC905, NTUB1, and OVCAR3) that could be substantially arrested at mitosis by ATO or Taxol (Fig. 4). On the other hand, the BubR1 or Mad2 level either was very low in untreated cells or was not accumulated after ATO treatment in cancer cell lines that could not be effectively arrested at mitosis by ATO or Taxol (Fig. 4). Since depletion of either BubR1 or Mad2 leads to attenuation of the spindle checkpoint function [Sudo et al., 2004], the low BubR1 protein level in cancer cell lines might therefore be sufficient to hamper the activation of spindle checkpoint in response to ATO or Taxol. In addition, our results were consistent with the notion that the function of spindle checkpoint proteins are regulated translationally and/or post-translationally by several oncogene products or tumor suppressors and that impaired spindle checkpoint function in cancer cells is often associated with a weakened responsiveness of the spindle checkpoint proteins to mitotic damages [Kops et al., 2005].

Our previous study showed that ATO-induced accumulation of BubR1 and Mad2 proteins and induction of mitotic arrest could be efficiently diminished by siRNA-mediated silencing of Mad2 or BubR1 [Yih et al., 2006]. The critical role of functional activation of the spindle checkpoint on Taxol- or ATO-induced mitotic arrest and apoptosis was therefore examined by siRNA-mediated silencing of Mad2 and BubR1 in CGL-4 and BFTC905, two cell lines highly sensitive to Taxol- or ATO-induced mitotic arrest and apoptosis. Significant reduction of BubR1 and Mad2 protein levels was confirmed by immunoblot analysis after siRNA transfection (Fig. 5A). Induction of mitotic arrest by a 24-h treatment of ATO or Taxol was dramatically reduced in BubR1 and Mad2 siRNA–transfected cells (Fig. 5B), showing that spindle checkpoint function was effectively inhibited after simultaneous knockdown of the two spindle checkpoint proteins. In addition, induction of apoptosis by a 48-h treatment of ATO or Taxol was also considerably decreased (Fig. 5C) and induction of micronucleated or multinucleated cells increased (Fig. 5D) in BubR1 and Mad2 siRNA–transfected cells compared with non-transfected or control siRNA–transfected cells. These results confirm that activation of the spindle checkpoint and induction of mitotic arrest are crucial for ATO-induced apoptosis and that no proper activation of the spindle checkpoint would lead to micronucleation or multi-nucleation in survived cells.

DISCUSSION

Our results show that ATO-induced mitotic arrest and apoptosis are significantly augmented in cancer cell lines with functional activation of the spindle checkpoint and that attenuation of spindle checkpoint function considerably reduces ATO-induced mitotic arrest and apoptosis and enhances micro- or multi-nucleation in survived cells. These results demonstrate that, in cancer cells lacking normal p53 function, the activation of the spindle checkpoint is crucial for ATO-induced apoptosis. Our findings place the mitotic spindle checkpoint in a central position in the apoptotic cell death pathways induced by ATO.

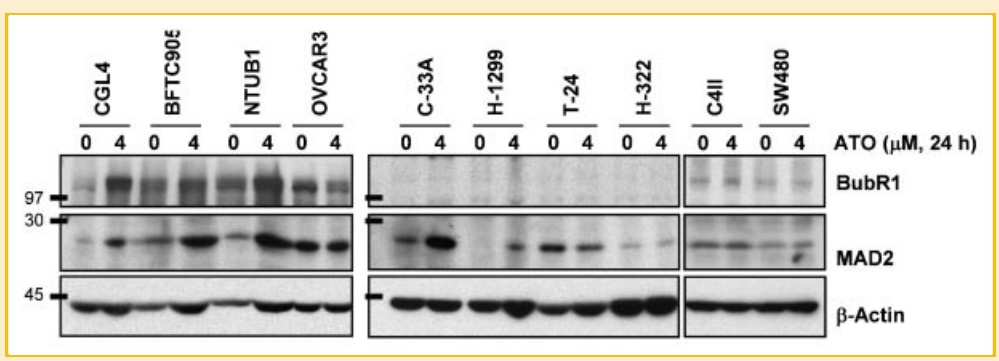

Fig. 4. The endogenous expression levels of BubR1 and Mad2 proteins in cancer cell lines. Logarithmically growing cells were **left** untreated or treated with 4 μM ATO for 24 h. All cells were collected and subjected for immunoblot analysis of BubR1 and Mad2. β-Actin was used as a loading control. The results are a representative of two independent experiments.

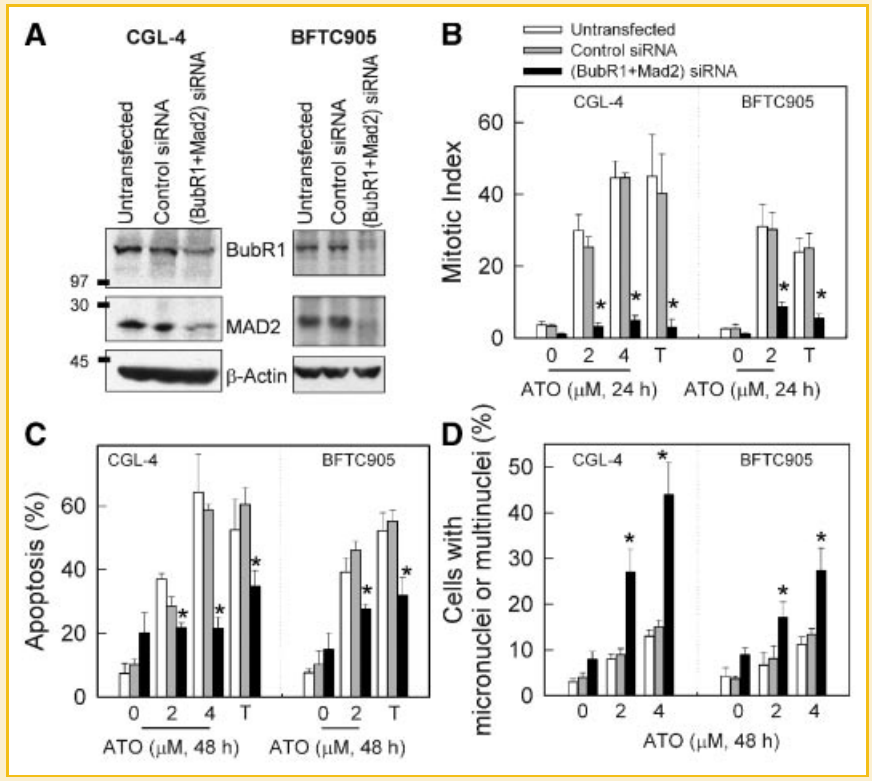

Fig. 5. Reduction of levels of the spindle checkpoint proteins BubR1 and Mad2 through RNA interference significantly reduces ATO- or Taxol-induced mitotic arrest and apoptosis in CGL-4 and BFTC905 cells. Cells were either not transfected or transfected with 50 nM control siRNA or BubR1 plus Mad2 siRNAs. A: Reduction of cellular BubR1 and Mad2 levels at 40 h after siRNA transfection by immunoblot analysis. At 24 h post-transfection with siRNA, the cells were treated with 2–4 μM ATO or 10 nM Taxol for 24 h and collected for analysis of mitotic arrest (B), treated for 48 h and collected for analysis of apoptosis (C), or treated for 48 h and fixed and stained with DAPI for analysis of micro- or multi-nucleation (D). The data represent the mean ± SD for three independent experiments. $^{*}P < 0.05$ compared with cells transfected with control siRNA and treated with Taxol or ATO, as appropriate.

Previous studies demonstrated that arsenite induces DNA damage, activates the p53-dependent DNA damage response, and arrests cell cycle progression at the G1 or G2 stage [Yih and Lee, 2000; Filippova and Duerksen-Hughes, 2003; Taylor et al., 2006]. It has also been shown that cells transfected with mutant p53 have increased sensitivity to arsenite compared with cells transfected with wild-type p53 [Salazar et al., 1997]. These results indicate that the p53-dependent cell cycle checkpoint may prevent arsenite-treated cancer cells from entering mitosis and that the induction of functional p53 confers resistance to arsenite. Furthermore, a number of genes involved in mitosis regulation are transcriptionally suppressed by p53 [Bhonde et al., 2006]. The high expression of mitotic genes in p53 mutant cells after DNA damage induction activates the spindle checkpoint and contributes to apoptosis, whereas their suppression in p53 wild type cells acts as a safeguard mechanism preventing mitosis initiation after DNA damage and the subsequent apoptosis [Bhonde et al., 2006]. Our present results demonstrating the critical role of the spindle checkpoint in arsenite-induced apoptosis were obtained from a panel of cancer cell lines lacking normal p53 function and therefore indicate that arsenite-induced apoptosis via spindle checkpoint activation is p53-independent and that loss of p53 function might be a pre-requisite for arsenite-induced mitotic arrest and apoptosis.

Our results demonstrated that activation of the spindle checkpoint and the resulting induction of mitotic arrest are critical determinants for ATO-induced apoptosis. The microtubule-disrupting drugs nocodazole, colcemid, Taxol, and vinblastine can activate the spindle checkpoint to arrest cells at mitosis. It has been reported that a dominant-negative mutant of Bub1 impairs the spindle checkpoint and allows cells to escape from apoptosis induced by nocodazole [Taylor and McKeon, 1997]. Altered localization of Mad1 and Mad2 induced by the Tax protein of human T cell leukemia virus type I correlates with loss of spindle checkpoint function and chemoresistance to microtubule-disrupting drugs [Kasai et al., 2002]. Sudo et al. [2004] demonstrated that suppression of Mad2 and BubR1 in Taxol-treated cancer cells abolished checkpoint function, resulting in Taxol resistance, whereas overexpression of Mad2 in cells with a checkpoint defect attributable to low Mad2 expression restored checkpoint function, resulting in enhanced Taxol sensitivity. In addition, use of small molecules that can inhibit the G2 DNA damage checkpoint in combination with DNA damaging agents also depends on a functional spindle checkpoint to induce mitotic arrest and mitotic cell death [Vogel et al., 2005]. These findings demonstrate a direct link between spindle checkpoint function and cellular sensitivity to microtubule-disrupting drug–induced apoptosis. Because arsenite alters the

dynamics of microtubule polymerization and causes mitotic spindle disorganization [Huang and Lee, 1998; Li and Broome, 1999; Ling et al., 2002], arsenite might thereby activate the spindle checkpoint, leading to mitotic arrest and hence apoptosis.

The signaling pathway linking the activation of the spindle checkpoint to the initiation of apoptosis remains controversial. Several reports revealed that, in response to mitotic spindle alterations induced by microtubule- or kinesin-targeting agents, mitotic slippage is preceded by the activation of the spindle checkpoint and followed by induction of apoptosis [Chen et al., 2003; Tao et al., 2005]. Others demonstrated that, after activation of the spindle checkpoint, mitotically arrested cells exit from mitosis, enter into a tetraploid G1 stage and then undergo apoptosis in a p53-dependent manner [Casenghi et al., 1999; Vogel et al., 2004]. However, in response to alterations of chromosome movement induced by depletion of the nuclear phosphoprotein mitosin, cells arrest at metaphase with multipolar spindles and lagging chromosomes and then die through apoptosis before initiation of anaphase [Yang et al., 2005]. Depletion of DNA damage checkpoint protein 1 leads to the accumulation of damaged DNA, premature activation of cyclin-dependent kinase 1, and concomitant induction of apoptosis [Niida et al., 2005]. In addition, the spindle checkpoint can be activated in response to chromosome and/or DNA damage during mitosis [Rieder et al., 1995; Morrison and Rieder, 2004]. Several recent studies have implicated incompletely replicated or damaged DNA during mitosis in the generation of centrosome aberrations and activation of the spindle checkpoint followed by mitotic cell death [Hut et al., 2003; Takada et al., 2003]. These results imply that, in the presence of damaged DNA or abnormally segregated chromosomes, apoptosis can be directly induced during mitotic arrest without mitotic slippage or exit. We have previously demonstrated the presence of damaged DNA in arsenite-arrested mitotic cells [Yih et al., 2005]. Centrosome amplification, which was also induced during the arsenite-induced mitotic arrest, may have initiated the subsequent apoptosis in a spindle checkpoint–dependent manner [Yih et al., 2006]. These results indicate that, in addition to microtubule dysfunction, arsenite-induced chromosome and/or DNA damage might also play a pivotal role in spindle checkpoint activation, induction of mitotic abnormalities, mitotic arrest, and mitotic apoptosis.

A number of reports have demonstrated that the spindle checkpoint is impaired in several types of cancer cells [Cahill et al., 1998; Wang et al., 2000, 2002; Tighe et al., 2001; Sze et al., 2004]. The defect in spindle checkpoint function may provoke further genomic instability [Kops et al., 2005], thereby altering the cellular response to anti-cancer drugs [Jordan and Wilson, 2004]. Arsenic compounds are known carcinogens. We have previously demonstrated that arsenite induces chromosome instability by altering the progression of mitosis [Yih et al., 1997]. In addition, arsenite-induced mitotic abnormalities can lead to mitotic arrest and then apoptosis [Yih et al., 2005, 2006]. Our results in this study demonstrated attenuation of the spindle checkpoint function not only reduce ATO-induced mitotic arrest and apoptosis but significantly increase the formation of micronuclei or multinuclei in survived cells. These results indicated that, if mitosis-mediated apoptosis is not triggered, then it may be inevitable that cells surviving arsenite-induced aberrant mitosis develop chromosome instability.

The present study clearly demonstrates a direct correlation between the induction of arsenite-induced apoptosis and the functional activation of the spindle checkpoint and resultant mitotic arrest in cancer cells lacking p53 function. ATO therefore might have better therapeutic effects on tumors with competent spindle checkpoint function and p53 mutations. However, considering that the spindle checkpoint is often impaired in cancer cells and that multiple factors could compromise spindle checkpoint function, the regulation of mitosis-mediated apoptosis warrants further attention and may be relevant to potential cancer therapeutics.

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REFERENCES

Akao Y, Mizoguchi H, Kojima S, Naoe T, Ohishi N, Yagi K. 1998. Arsenic induces apoptosis in B-cell leukaemic cell lines in vitro: Activation of caspases and down-regulation of Bcl-2 protein. Br J Haematol 102:1055–1060.

Bhonde MR, Hanski ML, Budczies J, Cao M, Gillissen B, Moorthy D, Simonetta F, Scherubl H, Truss M, Hagemeier C, Mewes HW, Daniel PT, Zeitz M, Hanski C. 2006. DNA damage-induced expression of p53 suppresses mitotic checkpoint kinase hMps1: The lack of this suppression in p53MUT cells contributes to apoptosis. J Biol Chem 281:8675–8685.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. 1998. Mutations of mitotic checkpoint genes in human cancers. Nature 392:300–303.

Cai X, Yu Y, Huang Y, Zhang L, Jia PM, Zhao Q, Chen Z, Tong JH, Dai W, Chen GQ. 2003. Arsenic trioxide-induced mitotic arrest and apoptosis in acute promyelocytic leukemia cells. Leukemia 17:1333–1337.

Casenghi M, Mangiacasale R, Tuynder M, Caillet-Fauquet P, Elhajouji A, Lavia P, Mousset S, Kirsch-Volders M, Cundari E. 1999. p53-independent apoptosis and p53-dependent block of DNA rereplication following mitotic spindle inhibition in human cells. Exp Cell Res 250:339–350.

Chen JG, Horwitz SB. 2002. Differential mitotic responses to microtubule-stabilizing and -destabilizing drugs. Cancer Res 62:1935–1938.

Chen GQ, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY, Jin XL, Tang W, Li XS, Xong SM, Shen ZX, Sun GL, Ma J, Zhang P, Zhang TD, Gazin C, Naoe T, Chen SJ, Wang ZY, Chen Z. 1996. In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood 88:1052–1061.

Chen GQ, Shi XG, Tang W, Xiong SM, Zhu J, Cai X, Han ZG, Ni JH, Shi GY, Jia PM, Liu MM, He KL, Niu C, Ma J, Zhang P, Zhang TD, Paul P, Naoe T, Kitamura K, Miller W, Waxman S, Wang ZY, de The H, Chen SJ, Chen Z. 1997. Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): I. As_2O_3 exerts dose-dependent dual effects on APL cells. Blood 89:3345–3353.

Chen JG, Yang CP, Cammer M, Horwitz SB. 2003. Gene expression and mitotic exit induced by microtubule-stabilizing drugs. Cancer Res 63:7891–7899.

Doherty SC, McKeown SR, McKelvey-Martin V, Downes CS, Atala A, Yoo JJ, Simpson DA, Kaufmann WK. 2003. Cell cycle checkpoint function in bladder cancer. J Natl Cancer Inst 95:1859–1868.

Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. 2001. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411:494–498.

Filippova M, Duerksen-Hughes PJ. 2003. Inorganic and dimethylated arsenic species induce cellular p53. Chem Res Toxicol 16:423–431.

Huang SC, Lee TC. 1998. Arsenite inhibits mitotic division and perturbs spindle dynamics in HeLa S3 cells. Carcinogenesis 19:889–896.

Huang SC, Huang CYF, Lee TC. 2000. Induction of mitosis-mediated apoptosis by sodium arsenite in HeLa S3 cells. Biochem Pharmacol 60:771–780.

Hut HM, Lemstra W, Blaauw EH, Van Cappellen GW, Kampinga HH, Sibon OC. 2003. Centrosomes split in the presence of impaired DNA integrity during mitosis. Mol Biol Cell 14:1993–2004.

Hyun ParkW, Hee Cho Y, Won Jung C, Oh Park J, Kim K, Hyuck Im Y, Lee MH, Ki Kang W, Park K. 2003. Arsenic trioxide inhibits the growth of A498 renal cell carcinoma cells via cell cycle arrest or apoptosis. Biochem Biophys Res Commun 300:230–235.

Jordan MA, Wilson L. 2004. Microtubules as a target for anticancer drugs. Nat Rev Cancer 4:253–265.

Kajiguchi T, Yamamoto K, Hossain K, Akhand AA, Nakashima I, Naoe T, Saito H, Emi N. 2003. Sustained activation of c-jun-terminal kinase (JNK) is closely related to arsenic trioxide-induced apoptosis in an acute myeloid leukemia (M2)-derived cell line, NKM-1. Leukemia 17:2189–2195.

Kasai T, Iwanaga Y, Iha H, Jeang KT. 2002. Prevalent loss of mitotic spindle checkpoint in adult T-cell leukemia confers resistance to microtubule inhibitors. J Biol Chem 277:5187–5193.

Kops GJ, Weaver BA, Cleveland DW. 2005. On the road to cancer: Aneuploidy and the mitotic checkpoint. Nat Rev Cancer 5:773–785.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Li YM, Broome JD. 1999. Arsenic targets tubulins to induce apoptosis in myeloid leukemia cells. Cancer Res 59:776–780.

Ling YH, Jiang JD, Holland JF, Perez-Soler R. 2002. Arsenic trioxide produces polymerization of microtubules and mitotic arrest before apoptosis in human tumor cell lines. Mol Pharmacol 62:529–538.

Liu Q, Hilsenbeck S, Gazitt Y. 2003. Arsenic trioxide-induced apoptosis in myeloma cells: p53-dependent G1 or G2/M cell cycle arrest, activatio o caspase-8 or caspase-9, and synergy with APO2/TRAIL. Blood 101:4078–4087.

Masuda A, Maeno K, Nakagawa T, Saito H, Takahashi T. 2003. Association between mitotic spindle checkpoint impairment and susceptibility to the induction of apoptosis by anti-microtubule agents in human lung cancers. Am J Pathol 163:1109–1116.

Mathas S, Lietz A, Janz M, Hinz M, Jundt F, Scheidereit C, Bommert K, Dorken B. 2003. Inhibition of NF-kappaB essentially contributes to arsenic-induced apoptosis. Blood 102:1028–1034.

Morrison C, Rieder CL. 2004. Chromosome damage and progression into and through mitosis in vertebrates. DNA Repair (Amst) 3:1133–1139.

Niida H, Tsuge S, Katsuno Y, Konishi A, Takeda N, Nakanishi M. 2005. Depletion of Chk1 leads to premature activation of Cdc2-cyclin B and mitotic catastrophe. J Biol Chem 280:39246–39252.

Ora I, Bondesson L, Jonsson C, Ljungberg J, Porn-Ares I, Garwicz S, Pahlman S. 2000. Arsenic trioxide inhibits neuroblastoma growth in vivo and promotes apoptotic cell death in vitro. Biochem Biophys Res Commun 277:179–185.

Rieder CL, Cole RW, Khodjakov A, Sluder G. 1995: The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitor signal produced by unattached kinetochores. J Cell Biol 130:941–948.

Salazar AM, Ostrosky Wegman P, Menendez D, Miranda E, Garcia Carranca A, Rojas E. 1997. Induction of p53 protein expression by sodium arsenite. Mutat Res 381:259–265.

Soignet SL, Maslak P, Wang ZG, Jhanwar S, Calleja E, Dardashti LJ, Corso D, DeBlasio A, Gabrilove J, Scheinberg DA, Pandolfi PP, Warrell RP, Jr. 1998. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med 339:1341–1348.

Stanbridge EJ, Flandermeyer RR, Daniels DW, Nelson-Rees WA. 1981. Specific chromosome loss associated with the expression of tumorigenicity in human cell hybrids. Somatic Cell Genet 7:699–712.

States JC, Reiners JJ, Jr., Pounds JG, Kaplan DJ, Beauerle BD, McNeely SC, Mathieu P, McCabe MJ, Jr. 2002. Arsenite disrupts mitosis and induces apoptosis in SV40-transformed human skin fibroblasts. Toxicol Appl Pharmacol 180:83–91.

Sudo T, Nitta M, Saya H, Ueno NT. 2004. Dependence of paclitaxel sensitivity on a functional spindle assembly checkpoint. Cancer Res 64:2502–2508.

Sze KM, Ching YP, Jin DY, Ng IO. 2004. Association of MAD2 expression with mitotic checkpoint competence in hepatoma cells. J Biomed Sci 11:920–927.

Takada S, Kelkar A, Theurkauf WE. 2003. Drosophila checkpoint kinase 2 couples centrosome function and spindle assembly to genomic integrity. Cell 113:87–99.

Takahashi T, Haruki N, Nomoto S, Masuda A, Saji S, Osada H, Takahashi T. 1999. Identification of frequent impairment of the mitotic checkpoint and molecular analysis of the mitotic checkpoint genes, hsMAD2 and p55CDC, in human lung cancers. Oncogene 18:4295–4300.

Tao W, South VJ, Zhang Y, Davide JP, Farrell L, Kohl NE, Sepp-Lorenzino L, Lobell RB. 2005. Induction of apoptosis by an inhibitor of the mitotic kinesin KSP requires both activation of the spindle assembly checkpoint and mitotic slippage. Cancer Cell 8:49–59.

Taylor SS, McKeon F. 1997. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. Cell 89:727–735.

Taylor BF, McNeely SC, Miller HL, Lehmann GM, McCabe MJ, Jr., States JC. 2006. P53 suppression of arsenite-induced mitotic catastrophe is mediated by p21CIP1/WAF1. J Pharmacol Exp Ther.

Tighe A, Johnson VL, Albertella M, Taylor SS. 2001. Aneuploid colon cancer cells have a robust spindle checkpoint. EMBO Rep 2:609–614.

Torres K, Horwitz SB. 1998. Mechanisms of Taxol-induced cell death are concentration dependent. Cancer Res 58:3620–3626.

Tzeng CC, Liu HS, Li C, Jin YT, Chen RM, Yang WH, Lin JS. 1996. Characterization of two urothelium cancer cell lines derived from a blackfoot disease endemic area in Taiwan. Anticancer Res 16:1797–1804.

Vogel C, Kienitz A, Hofmann I, Muller R, Bastians H. 2004. Crosstalk of the mitotic spindle assembly checkpoint with p53 to prevent polyploidy. Oncogene 23:6845–6853.

Vogel C, Kienitz A, Muller R, Bastians H. 2005. The mitotic spindle checkpoint is a critical determinant for topoisomerase-based chemotherapy. J Biol Chem 280:4025–4028.

Wang TS, Kuo CF, Jan KY, Huang H. 1996. Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. J Cell Physiol 169:256–268.

Wang ZG, Rivi R, Delva L, Konig A, Scheinberg DA, Gambacorti-Passerini C, Gabrilove JL, Warrell RP, Jr., Pandolfi PP. 1998. Arsenic trioxide and melarsoprol induce programmed cell death in myeloid leukemia cell lines and function in a PML and PML-RARalpha independent manner. Blood 92:1497–1504.

Wang X, Jin DY, Wong YC, Cheung AL, Chun AC, Lo AK, Liu Y, Tsao SW. 2000. Correlation of defective mitotic checkpoint with aberrantly reduced

expression of MAD2 protein in nasopharyngeal carcinoma cells. Carcinogenesis 21:2293–2297.

Wang X, Jin DY, Ng RW, Feng H, Wong YC, Cheung AL, Tsao SW. 2002. Significance of MAD2 expression to mitotic checkpoint control in ovarian cancer cells. Cancer Res 62:1662–1668.

Yang Z, Guo J, Chen Q, Ding C, Du J, Zhu X. 2005. Silencing mitosin induces misaligned chromosomes, premature chromosome decondensation before anaphase onset, and mitotic cell death. Mol Cell Biol 25:4062–4074.

Yih LH, Lee TC. 2000. Arsenite induces p53 accumulation through an ATM-dependent pathway in human fibroblasts. Cancer Res 60:6346–6352.

Yih LH, Lee TC. 2003. Induction of C-anaphase and diplochromosome through dysregulation of spindle assembly checkpoint by sodium arsenite in human fibroblasts. Cancer Res 63:6680–6688.

Yih LH, Ho IC, Lee TC. 1997. Sodium arsenite disturbs mitosis and induces chromosome loss in human fibroblasts. Cancer Res 57:5051–5059.

Yih LH, Hsueh SW, Luu WS, Chiu TH, Lee TC. 2005. Arsenite induces prominent mitotic arrest via inhibition of G2 checkpoint activation in CGL-2 cells. Carcinogenesis 26:53–63.

Yih LH, Tseng YY, Wu YC, Lee TC. 2006. Induction of centrosome amplification during arsenite-induced mitotic arrest in CGL-2 cells. Cancer Res 66:2098–2106.

Yu HJ, Tsai TC, Hsieh TS, Chiu TY. 1992. Characterization of a newly established human bladder carcinoma cell line, NTUB1. J Formos Med Assoc 91:608–613.

Zhang TC, Cao EH, Li JF, Ma W, Qin JF. 1999. Induction of apoptosis and inhibition of human gastric cancer MGC-803 cell growth by arsenic trioxide. Eur J Cancer 35:1258–1263.

Zheng Y, Shi Y, Tian C, Jiang C, Jin H, Chen J, Almasan A, Tang H, Chen Q. 2003. Essential role of the voltage-dependent anion channel (VDAC) in mitochondrial permeability transition pore opening and cytochrome c release induced by arsenic trioxide. Oncogene 1:1.

Zhu XH, Shen YL, Jing YK, Cai X, Jia PM, Huang Y, Tang W, Shi GY, Sun YP, Dai J, Wang ZY, Chen SJ, Zhang TD, Waxman S, Chen Z, Chen GQ. 1999. Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinically achievable concentrations. J Natl Cancer Inst 91:772–778.