

Radiation-induced abnormal centrosome amplification and mitotic catastrophe in human cervical tumor HeLa cells and murine mammary tumor EMT6 cells

Masaki Fujimoto, Tomoki Bo, Kumiko Yamamoto, Hironobu Yasui, Tohru Yamamori, and Osamu Inanami*

Laboratory of Radiation Biology, Department of Applied Veterinary Sciences, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

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Mitotic catastrophe is a form of cell death linked to aberrant mitosis caused by improper or uncoordinated mitotic progression. Abnormal centrosome amplification and mitotic catastrophe occur simultaneously, and some cells with amplified centrosomes enter aberrant mitosis, but it is not clear whether abnormal centrosome amplification triggers mitotic catastrophe. Here, to investigate whether radiation-induced abnormal centrosome amplification is essential for induction of radiation-induced mitotic catastrophe, centrinone-B, a highly selective inhibitor of polo-like kinase 4, was utilized to inhibit centrosome amplification, since polo-like kinase 4 is an essential kinase in centrosome duplication. When human cervical tumor HeLa cells and murine mammary tumor EMT6 cells were irradiated with 2.5 Gy of X-rays, cells with morphological features of mitotic catastrophe and the number of cells having >2 centrosomes increased in both cell lines. Although centrinone-B significantly inhibited radiation-induced abnormal centrosome amplification in both cell lines, such treatment did not change cell growth and significantly enhanced mitotic catastrophe in HeLa cells exposed to X-rays. In contrast, inhibition of centrosome amplification reduced cell growth and mitotic catastrophe in EMT6 cells exposed to X-rays. These results indicated that the role of radiation-induced abnormal centrosome amplification in radiation-induced mitotic catastrophe changes, depending on the cell type.

Key Words: mitotic catastrophe, centrosome, centrinone-B, radiation, polo-like kinase 4 (PLK4)

Mitotic catastrophe (MC) is a type of cell death induced during mitosis as a result of DNA damage, or by deranged spindle formation coupled to the insufficiency of different checkpoint mechanisms that would normally arrest the cell cycle before mitosis and hence suppress catastrophic events until repair has been achieved.^(1,2) Although tumor cells exposed to ionizing radiation are reported to die through different mechanisms including MC, senescence, necrosis and apoptosis,⁽³⁾ MC has been characterized as the main form of cell death induced in most non-hematopoietic tumor cells exposed to ionizing radiation.⁽⁴⁾

MC is generally considered to occur as a consequence of premature or improper entry of a cell into mitosis with radiation-induced DNA damage which could not be repaired at the G2/M or spindle checkpoints. It is generally considered that various regulatory proteins such as ATM, ATR, p53, Chk1, Chk2, polo-like kinase (PLK)1, PLK2, PLK3, Pin1, Mlh1 and 14-3-3-s⁽⁵⁾ are involved in MC. Our recent study⁽⁶⁾ demonstrated that MK-8776, a novel Chk1 inhibitor, induced radiosensitization though exacerbating MC and shortening the G2/M phase in murine mammary tumor EMT6 cells and human cervical carcinoma HeLa cells. On the

other hand, NMS-P715 and AZ3146, which are inhibitors of monopolar spindle 1 (MPS1), a serine/threonine kinase essential for the spindle assembly checkpoint, induce enhancement of cell killing and MC in EMT6 cells and murine squamous carcinoma SCCVII cells exposed to etoposide but not ionizing radiation.⁽⁷⁾ These results confirm the concept that radiation-induced G2/M checkpoint related to Chk1 is important in radiation-induced MC. Another factor for formation of MC is abnormal centrosome amplification. Dodson *et al.*⁽⁸⁾ reported that 60% of MC occurs in cells with >2 centrosomes in human osteosarcoma U2OS cells exposed to 2–10 Gy of γ -irradiation, suggesting a causal relationship between abnormal centrosome amplification and MC in irradiated tumor cells. However, it is not clear whether this relationship is universal phenomenon in tumor cells.

In this study, to investigate whether radiation-induced centrosome amplification is associated with radiation-induced MC, centrinone-B, a highly specific inhibitor of polo-like kinase (PLK) 4, which is an essential kinase for centriole duplication,⁽⁹⁾ was utilized to inhibit centrosome formation in human cervical tumor HeLa cells and murine mammary tumor EMT6 cells exposed to X-rays. In these cell lines, it is reported that p53 status of HeLa cells and EMT6 cells is loss of expression of p53⁽¹⁰⁾ and wild-type p53,⁽¹¹⁾ respectively. p53 status in cell lines may affect radiation-induced centrosome amplification, since recent report demonstrated that functional p53 controlled centromere protein A (CENP-A) through its chaperone, Holliday junction recognition protein (HJURP).⁽¹²⁾ Therefore, these two cell lines were picked up in this study. Furthermore, the role of centrosome amplification in radiation-induced MC was evaluated.

Materials and Methods

Reagents. Centrinone-B, a PLK4 inhibitor, was purchased from Tocris Bioscience (Bristol, UK). The nuclear stain 4',6-diamidino-2-phenylindole (DAPI) was obtained from Thermo Fisher Scientific (Waltham, MA). Anti γ -tubulin antibody and anti-PLK4 antibody were purchased from Sigma-Aldrich (St. Louis, MO) and Abcam Inc. (Cambridge, CA), respectively. Alexa Fluor[®] 488 anti-mouse IgG was obtained from Thermo Fisher Science.

Cell culture. Human cervical carcinoma HeLa cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific) with 10% (v/v) fetal bovine serum (FBS; Biosera, Nuaille, France) at 37°C in a humidified atmosphere of 5% CO₂. Murine breast cancer EMT6 cells were main-

*To whom correspondence should be addressed.
E-mail: inanami@vetmed.hokudai.ac.jp

tained RPMI1640 medium (Thermo Fisher Scientific) supplemented with 10% (v/v) fetal bovine serum at 37°C in a humidified atmosphere of 5% CO₂.

Evaluation of cytotoxicity to centrinone-B. Cytotoxicity to centrinone-B was performed according to the method described by Kueng *et al.*⁽¹³⁾ with some modifications. Briefly, HeLa cells (1×10^4 cells/well) and EMT6 cells (8×10^2 cells/well) were seeded in 24 well plate, respectively. After overnight culture, 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 μ M of centrinone-B was added each well, respectively, and then cells were cultured for 96 h. After removing culture medium, 1 ml of methanol was added to the well of plate for fixation, and plates were air-dried and stained by addition of 500 μ l of crystal violet staining solution (0.1% solution of crystal violet dissolved in 25% methanol) and incubation for 1 h. Excess dye was removed by extensive washing with distilled water and plates were air-dried prior to solubilization of bound dye in 1 ml methanol. The optical density of dye extracts was measured directly in plates using Victor Nivo™ multimode plate reader (PerkinElmer, Waltham, MA). The wavelength selected was 595 nm.

Immunohistochemistry of staining for PLK4. Cells were irradiated with 2.5 Gy of X-rays and then incubated for 48 h for HeLa cells and 24 h for EMT6 cells, respectively. Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min at room temperature. After being permeabilized with PBS containing 0.5% Triton X-100 for 5 min at 4°C, cells were blocked by treatment with PBS containing 6% bovine serum albumin (BSA) for 60 min at room temperature. The blocked cells were incubated with a goat anti-PLK4 antibody at 1:1,000 dilution in 6% BSA overnight at 4°C and then incubated in the dark with an Alexa Fluor 488-conjugated anti-goat secondary antibody at a 1:1,000 dilution for 90 min. After incubation, they were mounted with Prolong Gold antifade reagent (Thermo Fisher Scientific). Fluorescence microscopic analysis was performed using an Olympus BX61 microscope (Olympus, Tokyo, Japan) with reflected light fluorescence.

Centrinone-B treatment and X-irradiation. HeLa cells (6.25×10^4 cells) and EMT6 cells (1.0×10^4 cells) were seeded in 60 mm ϕ dishes with a cover slip, respectively. The confluency in both cell culture was extremely low (less than 10%). In a short-term experiment within 24 h, about 70% confluency of cell is usually selected in initial seeding. However, present experiments require a long observation period of up to 6 days and the growth rate of each cell is so fast. Thus, to avoid being too high confluent, or contact inhibition at the time of detection, the cell number at initial seeding was chosen to be extremely low density. After incubation for 24 h, centrinone-B was added and incubated 72 h in HeLa cells and 96 h in EMT6 cells as shown in Fig. 3A and B, and then irradiation with 2.5 Gy of X-rays was performed using an X-RAD iR-225 (Precision X-Ray, North Branford, CT) with a dose rate of 1.37 Gy/min at 200 kVp, 15 mA, with a 1.0 mm aluminum filter.

Analysis of MC and centrosome number. Analysis of MC and centrosome number was performed as previously described.^(7,14) At the indicated time after centrinone-B treatment and X-irradiation as shown in Fig. 3A and B, cells on glass coverslips were fixed with ice-cold methanol for 10 min at room temperature. After permeabilization with ice-cold acetone for 1 min at room temperature, the cells were washed three times with PBS, followed by treatment with PBS containing 6% (v/v) goat serum (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) for 1 h at room temperature. Subsequently, they were incubated with anti γ -tubulin antibody (1:5,000) in 3% (v/v) goat serum/PBS overnight at 4°C. Next, they were incubated in the dark with Alexa Fluor® 488 anti-mouse IgG (1:2,000) in 3% (v/v) goat serum/PBS for 1.5 h at room temperature. After incubation, they were washed three times with PBS and counterstained with 300 nM DAPI for 5 min at room temperature. The coverslips were mounted with ProLong® Gold Antifade Mountant reagent (Life Technologies, Carlsbad, CA).

Fluorescence microscopy analysis was performed with an Olympus BX61 microscope (Olympus) with reflected light fluorescence. For MC analysis, at least 100 cells were counted and cells with features of aberrant mitotic nuclei (micronuclei, multilobular and fragmented nuclei) were scored as cells undergoing MC. For centrosome analysis, the number of γ -tubulin foci per cell was counted manually. At least 100 cells were analyzed and the percentage of the cells containing more than two foci was determined.

Analysis of cell growth. Cells were seeded in 60 mm ϕ dishes and cultured overnight. Subsequently, the cells were cultured with or without 0.5 or 2.5 μ M of centrinone-B for 3 or 4 days, as shown in Fig. 3A and B. After X-irradiation, the number of cells was counted.

Statistical analysis. All results are expressed as mean \pm SD of three separate experiments. The variance ratio was estimated using the F-test, and comparison of two groups was performed using Student's *t* test or Welch's *t* test. For multiple comparisons, Dunnett's test was performed. The minimum level of significance was set at $p < 0.05$.

Results

X-irradiation-induced MC and abnormal centrosome amplification. In HeLa cells at 48 h after irradiation with 2.5 Gy of X-rays, DAPI staining and immunostaining with anti γ -tubulin showed that X-irradiation induced increased number of cells with features of MC such as micronuclei, multilobular or fragmented nuclei, and cells containing more than two centrosomes, respectively. Typical microphotographs are depicted in Fig. 1A. Quantitative analysis showed that the number of cells having the characteristics of MC was significantly increased at 12 h and later after 2.5 Gy of X-irradiation of HeLa (Fig. 1B) and EMT6 cells (Fig. 1C). On the other hand, cells containing more than two centrosomes were also significantly increased at 24 h and later after X-irradiation of HeLa cells (Fig. 1D), and 15 h later in EMT6 cells (Fig. 1E). These results suggested that radiation-induced MC and abnormal centrosome amplification occur at the same time. In addition, immunostaining images in X-irradiated HeLa cells (Fig. 1F) and EMT6 cells (Fig. 1G) revealed that PLK4 formed multiple aggregates in the nucleus of X-irradiated both cell lines with centrosome formation, indicating that activation of PLK4 was induced by X-irradiation.

Cytotoxicity to centrinone-B and effect of centrinone-B on centrosome amplification. Figure 3A and C showed dose-dependent cytotoxicity of centrinone-B in HeLa cells and EMT6 cells. In HeLa cells, treatment of concentrations from 0.1 to 0.5 μ M had little effect on surviving fraction, but toxicity was clearly observed above 1 μ M. On the other hand, significant cytotoxicity in EMT6 cells started at more high-dose (2.5 μ M) of centrinone-B. Next, effect of centrinone-B on centrosome formation was examined. In HeLa cells treated with 0.5 and 1 μ M centrinone-B (Fig. 2B), time-dependent increase of cells which had no centrosome was observed. Whereas, 0.25 μ M centrinone-B did not induce cells without centrosomes, and unexpectedly caused abnormal centrosome amplification. This unexpected result indicated that 0.25 μ M centrinone-B could not sufficiently inhibit the activity of PLK4 and induce unknown side effect such as the other pathway for activation of centrosome amplification in HeLa cells. In EMT6 cells (Fig. 2D), 1 and 2.5 μ M centrinone-B increased cells without centrosomes, though 2.5 μ M centrinone-B induced a few abnormal centrosome amplifications at 4 days after drug treatment. From these data, 0.5 and 1 μ M for HeLa cells and 1 and 2.5 μ M for EMT6 cells were suited to evaluate relationship between radiation-induced abnormal centrosome amplification and MC. Since HeLa cells are relatively sensitive in compared to EMT6 cells, the treatment time (72 h) of HeLa cells was selected to be shorter than that (96 h) of EMT6 cells for further analysis.

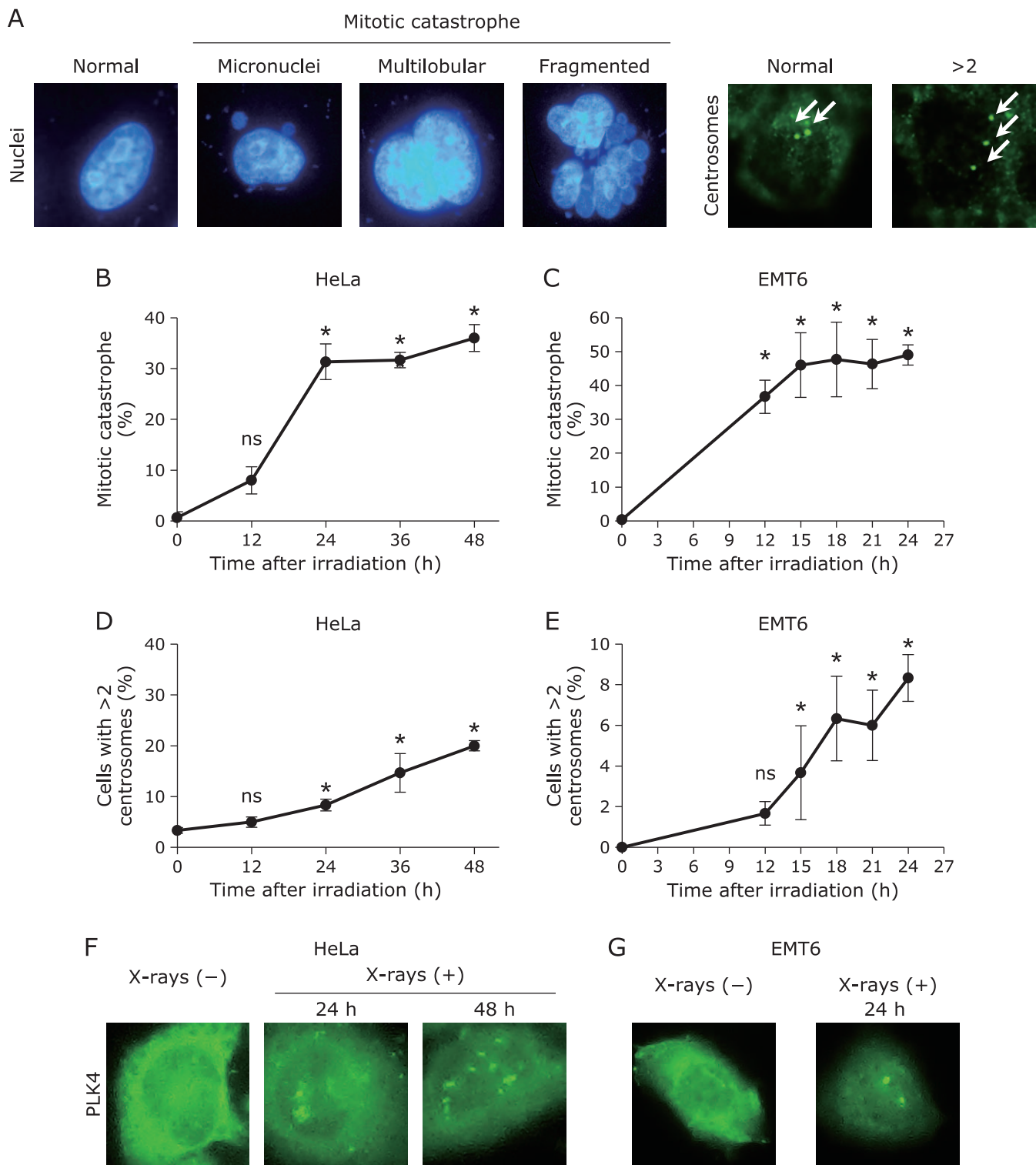


Fig. 1. Radiation-induced MC and abnormal centrosome amplification. MC and number of centrosomes were analyzed by DAPI staining and immunostaining with anti- γ -tubulin. (A) HeLa cells with features of mitotic catastrophe, such as micronuclei, multilobular and fragmented nuclei (left) and containing >2 centrosomes (right) 48 h after X-irradiation of 2.5 Gy. (B, D) MC and abnormal centrosome amplification were quantitated at 0, 12, 24, 36 and 48 h after X-irradiation of 2.5 Gy to HeLa cells. (C, E) MC and abnormal centrosome amplification were quantitated at 0, 12, 15, 18, 21 and 24 h after X-irradiation of 2.5 Gy to EMT6 cells. Data are expressed as mean \pm SD from three experiments. * p <0.05, ns, not significant (Dunnett's test). Representative immunostaining images of PLK4 expression and localization in (F) HeLa cells 24 and 48 h after X-irradiation and EMT6 cells at 24 h after X-irradiation.

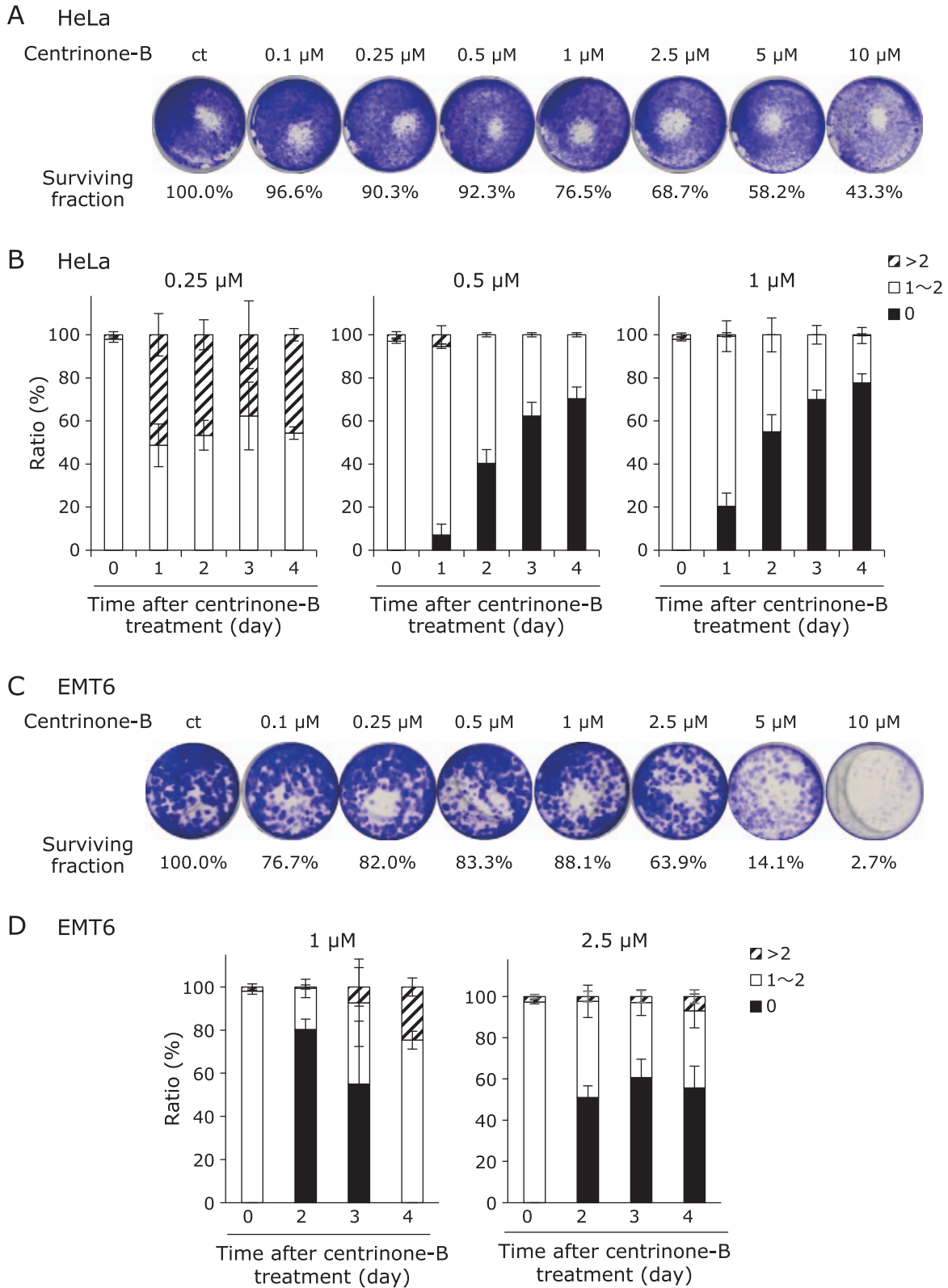


Fig. 2. Cytotoxicity to centrinone-B and effect of centrinone-B on centrosome formation. Cytotoxicity to centrinone-B in (A) HeLa cells and (C) EMT6 cells was analyzed by the method for cell number measurement in monolayer cultures by crystal violet staining described in Materials and Methods. Each survival fraction was expressed as a percentage of the absorbance at 595 nm of the sample treated with centrinone-B relative to the control (ct) sample not treated with centrinone-B. (B, D) Quantitative analysis of effect of centrinone-B on centrosome formation. (B) HeLa cells and (D) EMT6 cells were exposed to centrinone-B and incubated for 4 days in normal condition. The number of cells with abnormal centrosome amplification (cells with >2 centrosomes), cells with normal centrosomes (cells with 1 or 2 centrosomes) and cells without centrosomes were counted. The cell number was measured every 24 h until 4 days after addition of centrinone-B. Data was expressed as a percentage against total cell number.

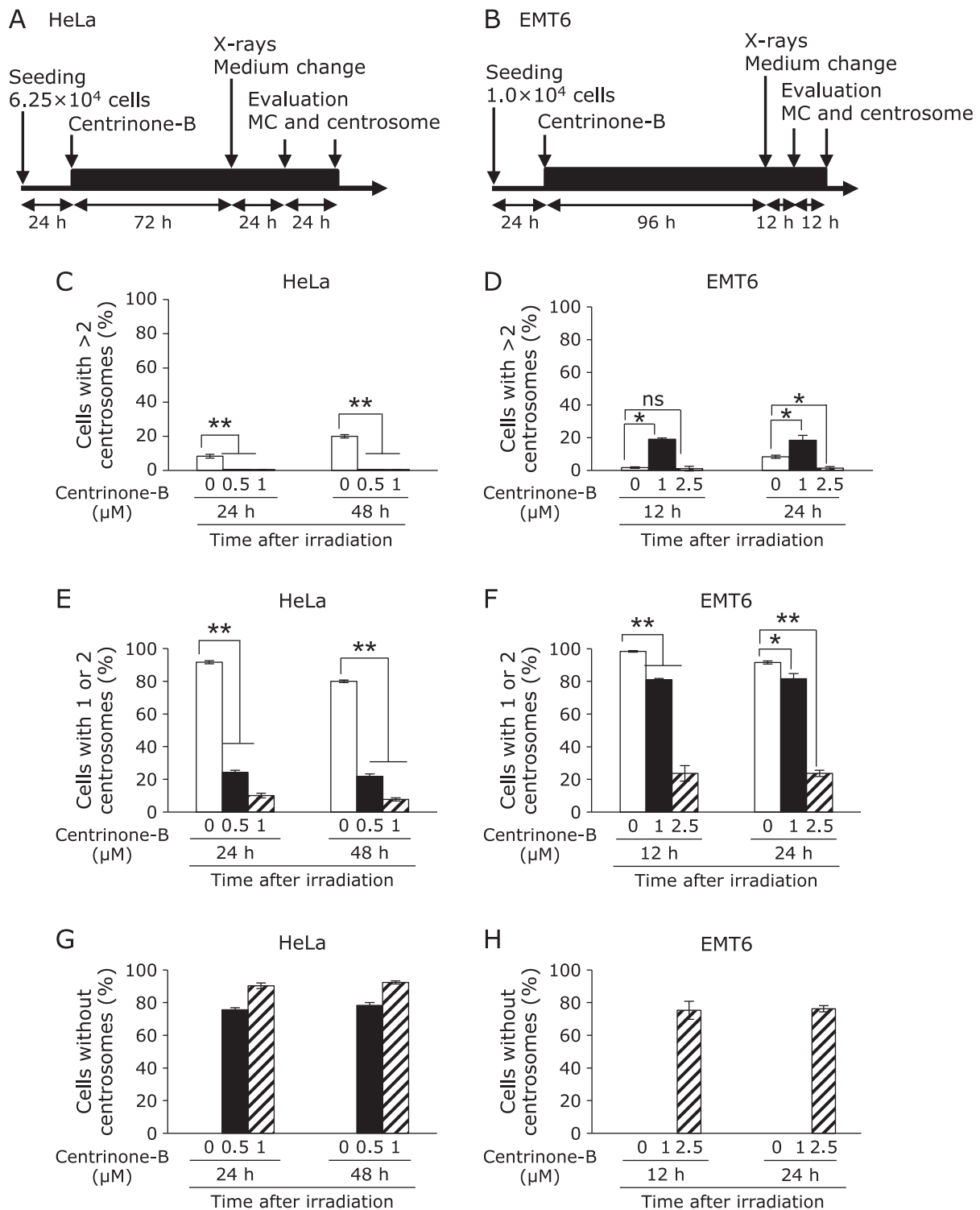


Fig. 3. Effect of PLK4 inhibitor on radiation-induced abnormal centrosome amplification. (A) HeLa cells were cultured for 72 h with 0.5 or 1 μM centrinone-B and then X-irradiated with 2.5 Gy. The number of centrosomes were analyzed by immunohistochemical staining at 24 and 48 h after X-irradiation. (B) EMT6 cells were cultured for 96 h after 1 μM or 2.5 μM centrinone-B and then X-irradiated with 2.5 Gy. Cells were MC and the number of centrosomes was analyzed 12 and 24 h after X-irradiation. (C–H) Effects of centrinone-B on centrosome formation were analyzed by immunostaining. HeLa cells were cultured without (white column) centrinone-B, with (black column) 0.5 μM and (shaded column) 1 μM centrinone-B before X-irradiation of 2.5 Gy. EMT6 cells were cultured without (white column), with (black column) 1 μM and (shaded column) 2.5 μM centrinone-B before X-irradiation of 2.5 Gy. At indicated time after irradiation, the number of cells with abnormal centrosome amplification (cells with >2 centrosomes), normal centrosomes (cells with 1 or 2 centrosomes) and cells without centrosomes were counted in (C, E, G) HeLa cells and (D, F, H) EMT6 cells. Data are expressed as mean ± SD from three experiments. **p*<0.05, ***p*<0.01 and ns, not significant (Student's *t* test).

Centrinone-B, a PLK4 inhibitor, inhibited radiation-induced abnormal centrosome amplification. Centrinone-B is reported to be a reversal inhibitor of PLK4, which is essential for centrosome duplication. It is reported to take at least two days for centrosome depletion after treatment with centrinone-B in HeLa cells and human fibroblast NIH3T3 cells.⁽⁹⁾ In this experiment, long-term pretreatment with centrinone-B from 3 to 4 days and then evaluation of MC formation from 12 to 48 h after irradiation was chosen as an experimental protocol to obtain information concerning the fate of cells during one or two rounds of cell cycling after irradiation, as shown in Fig. 3A and B. Briefly, cells were seeded and cultured for 24 h, treated with centrinone-B, and then irradiated after 3 days of centrinone-B treatment (HeLa cells), or after 4 days of centrinone-B treatment (EMT6 cell). MC and centrosome amplification were measured at 24 and 48 h in HeLa cells and 12 and 24 h in EMT6 cells after X-irradiation by taking the doubling time of cells into account. In this experiment, the doubling time for HeLa and EMT6 cells used was approximately 20 and 12.5 h, respectively.

In HeLa cells, 0.5 and 1 μ M centrinone-B treatment significantly inhibited radiation-induced abnormal centrosome amplification (cells with >2 centrosomes) (Fig. 3C) and cells with normal centrosomes (cells with 1 or 2 centrosomes) (Fig. 3E), and increased cells without centrosomes (Fig. 3G). In contrast, in EMT6, 2.5 μ M centrinone-B significantly reduced radiation-induced abnormal centrosome amplification (Fig. 3D) and increased cells without centrosomes (Fig. 3H) at 24 h after X-irradiation, whereas 2.5 μ M centrinone-B significantly reduced cells with normal centrosomes (Fig. 3F), suggesting that centrinone-B inhibited radiation-induced PLK4. Surprisingly, 1 μ M centrinone-B increased abnormal centrosome amplification at 12 and 24 h after irradiation (Fig. 3D) but did not increase cells without centrosomes (Fig. 3H). These results indicated that 0.5 μ M centrinone-B in HeLa and 2.5 μ M centrinone-B in EMT6 were necessary and sufficient to inhibit radiation-induced centrosome amplification.

Centrinone-B enhanced radiation-induced MC in HeLa cells, but inhibited it in EMT6 cells. Next, to determine the relationship between radiation-induced abnormal centrosome amplification and MC formation, the effect of inhibition of abnormal centrosome amplification by centrinone-B on radiation-induced MC was evaluated. Centrinone-B treatment enhanced radiation-induced total MC at 48 h after exposure to X-rays in HeLa cells (Fig. 4A), but inhibited radiation-induced MC at 12 and 24 h after exposure to X-rays in EMT6 cells (Fig. 4B). It seemed that formation of fragmented nuclei contributed to radiation-induced increase of total MC in HeLa cells and decrease of micronuclei contributed to radiation-induced decrease of MC in EMT6 cells. In contrast, centrinone-B did not influence the growth rate of HeLa cells exposed to X-rays (Fig. 4C), but significantly decreased the growth rate of EMT6 cells similarly exposed (Fig. 4D), indicating that abnormal centrosome amplification inhibits radiation-induced MC in HeLa cells, but induces it in EMT6 cells. These results suggested that the role of centrosome amplification in radiation-induced MC is dependent on cell type differences.

Discussion

Recently, there have been many reports concerning the therapeutic strategy of targeting PLK4,⁽¹⁵⁻¹⁷⁾ PLK2^(18,19) and Aurora kinase⁽²⁰⁾ to inhibit centrosome amplification in tumor cells, because centrosome defects have been found in pre-neoplastic and tumor tissues from breast, ovarian, prostate, head and neck, lung, liver, and bladder malignancies, among many others⁽¹⁷⁾ and abnormal centrosome amplification due to overexpression of PLK4 has been reported in tumor cells.⁽²¹⁾ In the present experiments, it was clearly demonstrated that inhibition of radiation-induced centrosome amplification by centrinone-B, a PLK4 inhibitor, promoted

MC without inhibition of cell growth in HeLa cells. On the other hand, centrinone-B was shown to inhibit not only radiation-induced centrosome amplification, but also MC and cell growth in EMT6 cells. This is the first demonstration of the effects of a PLK4 inhibitor on radiation-induced MC formation in tumor cells, and the results indicated that centrosome amplification caused by radiation has different effects on cell growth rate and MC depending on the cell type.

In general, in tumor cells, radiation-induced MC is at least partly originated from centrosome amplification, leading to multipolar mitosis and micronuclei,⁽⁴⁾ suggesting that inhibition of centrosome amplification attenuates not only cell growth but also radiation-induced MC. Mc Gee has described that MC senses mitotic damage and directs the defective cell to one of three possible antiproliferative fates such as death in M phase and cell death in G1 and senescence:⁽²²⁾ defective cells undergo death in mitosis in the presence of cyclin B, or cyclin B levels will gradually fall allowing the cells to undergo M-phase slippage and exit mitosis, where they subsequently undergo death in G1. Alternatively, cells can undergo senescence following slippage. In the present experiments involving EMT6 cells, radiation-induced MC was associated with radiation-induced centrosome amplification, and this phenomenon may be explained by centrosome-amplification-dependent MC formation to immediately prompt cell death in M or G1 phase.

Surprisingly, our present data involving HeLa cells showed that centrinone-B promoted radiation-induced MC without any influence on cell division during one or two rounds of cell cycle after irradiation, as shown in Fig. 4A and C, indicating that radiation-induced abnormal centrosome amplification had an inhibitory role in radiation-induced MC formation. However, it is generally considered that DNA damage-induced abnormal centrosome amplification is a mechanism for ensuring the death of cells that evade the DNA-damage or spindle assembly checkpoints,⁽⁵⁾ since the formation of MC occurs as a result of aberrant mitosis and will progress to cell death or senescence. In contrast to this general consideration, our data indicated that radiation-induced centrosome amplification may be a remarkable survival strategy in HeLa cells. A recent study using the fluorescent ubiquitination-based cell-cycle indicator (Fucci) showed that cell-cycle kinetics in HeLa cells after irradiation is quite unique, because a majority of HeLa cells irradiated with 5 Gy underwent almost normal mitotic segregation, at least in the first M phase, and endoreduplication rarely occurs,⁽²³⁾ although it was reported that multiploidy by endoreduplication is frequently induced in p53-mutated cells such as mouse mammary epithelial NMuMG cells⁽²⁴⁾ and p53 status affected to expressions of CENP-A and its chaperone, HJURP, related with chromosome segregation in mouse fibroblast cells.⁽¹²⁾ It remains unclear why this exceptional cell cycle behavior occurs in HeLa cells, in which p53 is non-functional.⁽¹⁰⁾ However, the survival strategy of HeLa cells at checkpoints following DNA damage may be different from other p53-mutated tumor cells. In this stage, it is unclear why the effect of the inhibition of radiation-induced abnormal centrosome amplification on MC formation was reverse depending on cell species. although there is a possibility that cell type difference is associated with the difference in repair process at the G2 checkpoint and spindle checkpoint in M phase. To further clarify the precise mechanisms involved in the radiation-induced increase in MC by centrinone-B in HeLa cells and EMT6 cells, observations using time-lapse imaging of single cells for longer periods will be necessary.

In summary, the present study demonstrated that radiation-induced abnormal centrosome amplification in radiation-induced MC changes depending on the cell type involved. These data are important for the development of combination radiotherapy with PLK4 inhibitor supplementation.

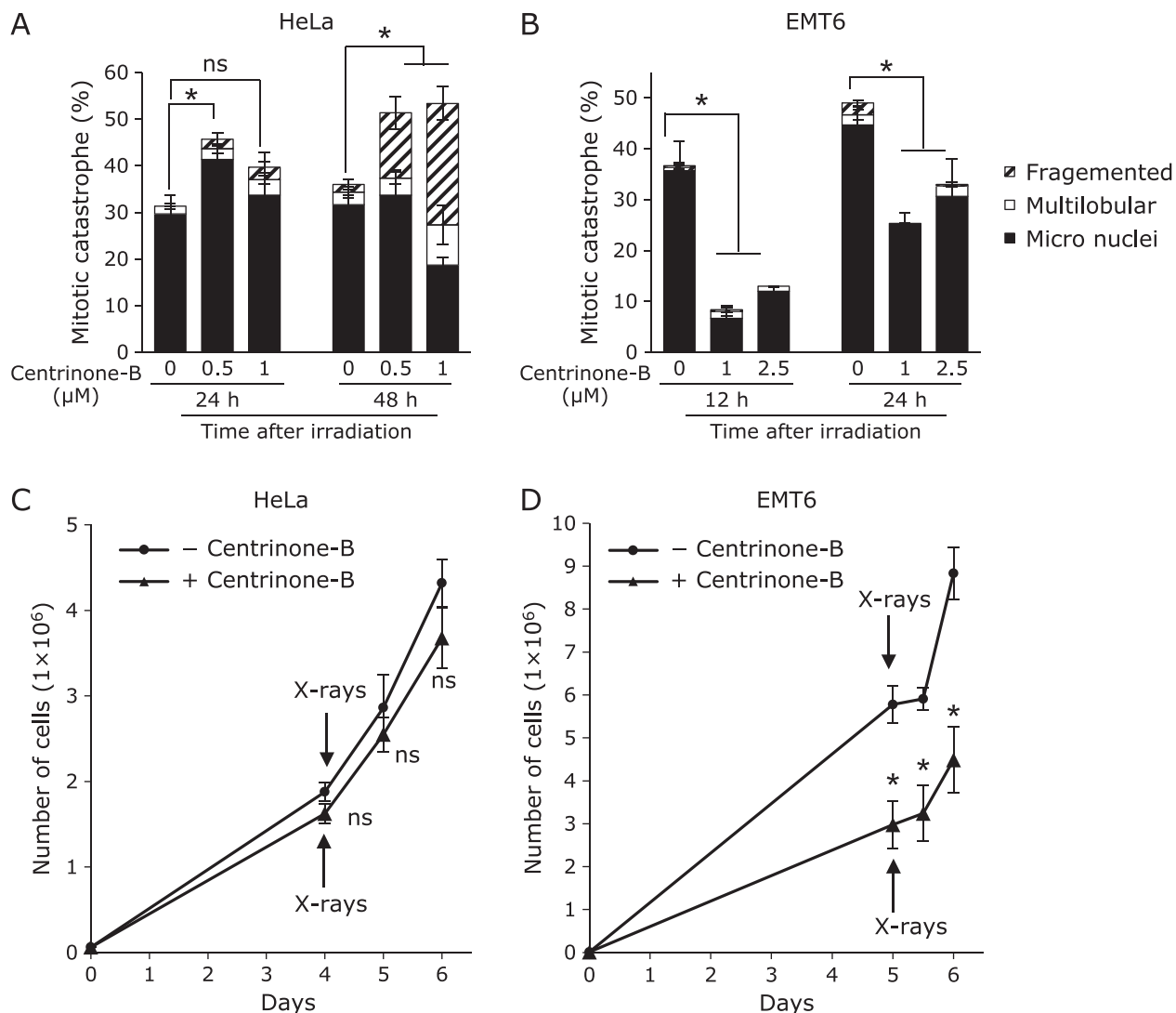


Fig. 4. Effect of centrinone-B, a PLK4 inhibitor, on radiation-induced MC. (A, B) Effects of centrinone-B on radiation-induced MC were analyzed by DAPI staining. (A) HeLa cells were cultured without centrinone-B, with 0.5 μM centrinone-B or 1 μM centrinone-B for 72 h before X-irradiation of 2.5 Gy. MC was quantitated at 24 h and 48 h after X-irradiation. MC is classified into three categories, (black column) micronuclei, (white column) multilobular nuclei and (shaded column) fragmented nuclei. (B) EMT6 cells were cultured without centrinone-B, with 1 μM centrinone-B or 2.5 μM centrinone-B for 96 h before X-irradiation of 2.5 Gy. MC was quantitated at 12 and 24 h after X-irradiation. MC is classified into follow three categories, (black column) micronuclei, (white column) multilobular nuclei and (shaded column) fragmented nuclei. Data are expressed as mean ± SD from three experiments. * $p < 0.05$ (Student's *t* test). (C, D) Effects of centrinone-B on cell growth were analyzed by counting cell number. (C) HeLa cells were cultured for 24 h and treated (▲) or not (●) with 500 nM centrinone-B for 3 days before X-irradiation of 2.5 Gy. The number of cells were counted at 0, 24 and 48 h after X-irradiation. (D) EMT6 cells were cultured for 24 h and treated (▲) or not (●) with 2.5 μM centrinone-B for 4 days before X-irradiation of 2.5 Gy. The number of cells were counted at 0, 12 and 24 h after X-irradiation. Data are expressed as mean ± SD from three experiments. * $p < 0.05$, ns, not significant (Dunnett's test).

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Abbreviations

BSA bovine serum albumin
 CENP-A centromere protein A
 DAPI 4',6-diamidino-2-phenylindole
 HJURP Holliday junction recognition protein

MC mitotic catastrophe
 MPS1 monopolar spindle 1
 PBS phosphate-buffered saline
 PLK polo-like kinase
 SAC spindle-check-point

Conflict of Interest

No potential conflicts of interest were disclosed.

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