Biochemical and Biophysical Research Communications 428 (2012) 224–229

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Unusual expression of red fluorescence at M phase induced by anti-microtubule agents in HeLa cells expressing the fluorescent ubiquitination-based cell cycle indicator (Fucci)

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ARTICLE INFO

Article history:
Received 1 October 2012
Available online 9 October 2012

Keywords:
Anti-microtubule agents
Fluorescent ubiquitination-based cell cycle indicator (Fucci)
Mitosis
Ubiquitination
Mitotic catastrophe

ABSTRACT

Plinabulin (NPI-2358) is a novel microtubule-depolymerizing agent. In HeLa cells, plinabulin arrests the cell-cycle at M phase and subsequently induces mitotic catastrophe. To better understand the effects on this compound on the cell-cycle, we used the fluorescent ubiquitination-based cell cycle indicator (Fucci), which normally enables G1 and S/G2/M cells to emit red and green fluorescence, respectively. When HeLa-Fucci cells were treated with 50 nM plinabulin, cells began to fluoresce both green and red in an unusual pattern; most cells exhibited the new pattern after 24 h of treatment. X-irradiation efficiently induced G2 arrest in plinabulin-treated cells and significantly retarded the emergence of the unusual pattern, suggesting that entering M phase is essential for induction of the pattern. By simultaneously visualizing chromosomes with GFP-histone H2B, we established that the pattern emerges after nuclear envelope breakdown but before metaphase. Pedigree assay revealed a significant relationship between the unusual expression and mitotic catastrophe. Nocodazole, KPU-133 (a more potent derivative of plinabulin), and paclitaxel also exerted similar effects. From these data, we conclude that the unusual pattern may be associated with dysregulation of late M phase-specific E3 ligase activity and mitotic catastrophe following treatment with anti-microtubule agents.

1. Introduction

Microtubules, which consist of α- and β-tubulin, are major components of the cytoskeleton and play roles in a plethora of cellular functions [1–3]. Among these functions, the regulation of chromosome separation via dynamic polymerization and depolymerization of microtubules in mitosis has been an important target of cancer therapy [3]. Numerous anti-microtubule agents have been developed; these compounds can be classified into three types, based on whether they bind β-tubulin at the taxan site, the vinca domain, or the colchicine site [1–3]. Agents belonging to the former two types, such as paclitaxel (microtubule-stabilizing agent) and vincristine (microtubule-depolymerizing agent), have been used extensively as chemotherapeutic agents, whereas colchicine (microtubule-depolymerizing agent) has not been approved due to its toxicity [4]. However, several agents with colchicine-like microtubule-depolymerizing activities have recently been developed; these compounds could serve as vascular disrupting agents as well as exerting cytotoxic effects directly on tumor cells themselves [4–6].

Plinabulin (NPI-2358/KPU-2) is a synthetic analog of the natural diketopiperazine phenylahistin, which has colchicine-like microtubule depolymerization activity [7,8]. A phase 1 study of plinabulin revealed that this compound has favorable pharmacokinetics, pharmacodynamics, and safety profile [9]; a phase 2 study carried out in four countries including the United States has yielded encouraging data regarding efficacy [4]. Because only a few reports have described the effects of plinabulin on cell-cycle events [4,8,10], we characterized these effects using the fluorescent ubiquitination-based cell cycle indicator (Fucci). Cells expressing Fucci normally emit red fluorescence in G1 and green fluorescence in S/G2/M phase [11], allowing visualization of the cell-cycle. Here we show that plinabulin induces abnormal expression of red fluorescence in M phase before mitotic catastrophe, suggesting that the ubiquitin system may be dysregulated at the stage, resulting in activation of cell-death pathways.

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http://dx.doi.org/10.1016/j.bbrc.2012.10.014

2. Materials and methods

2.1. Cell lines and culture conditions

HeLa cells expressing the Fucci probes [11] were provided by the RIKEN BRC through the National Bio-Resource Project of MEXT, Japan. HeLa cells stably expressing GFP-tagged histone H2B [12] were kindly provided by Dr. H. Saya (Keio University). All cells were maintained in DMEM (Sigma–Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO_2.

2.2. Treatments

Cells were seeded onto 60-mm or 35-mm dishes and subcultured for at least 10 h before being treated for the indicated times with plinabulin, 5 nM KPU-133 (16j in Ref. [4]), 50 nM paclitaxel (Wako, Osaka, Japan), or 50 ng/mL nocodazole (Wako). Cells were also irradiated using an RX650 X-ray cabinet system (130 kV, 5 mA, 0.5 mm Al filtration) (Faxitron, Tucson, AZ) at a dose rate of 0.8 Gy/min.

2.3. Transduction

To visualize chromosomes, we used CellLight™ BacMam 2.0 (Life Technologies, Carlsbad, CA). The BacMam technology uses baculovirus as a vehicle for transducing genes into mammalian cells. We transduced the human histone H2B gene fused to the gene encoding green fluorescent protein (GFP) into HeLa cells expressing the Fucci probes, according to the manufacturer's protocol. Briefly, cells (5×10^4 cells/well) were seeded onto CELLview Glass Bottom Dish chamber slides (Greiner Bio-One, Frickenhausen, Germany) and subcultured overnight. An aliquot (7.5 μL/well) of CellLight™ Histone 2B reagent was added to the medium and cultured for at least 16 h before plinabulin treatment.

2.4. Flow cytometric analysis

Two types of samples were subjected to flow-cytometric analysis: non-fixed samples for detecting fluorescence intensity of mAG or mKO2, and fixed samples for DNA content analysis. After each treatment, collected culture medium and trypsinized cells were centrifuged together, and the pellets were washed in ice-cold PBS. For DNA content analysis, cells were fixed in ice-cold 70% ethanol in PBS for at least 30 min on ice. After fixation, cells were rewashed in ice-cold PBS and incubated in 0.5 g/mL 7-AAD solution (BD Bioscience, Franklin Lakes, NJ). Finally, both non-fixed and fixed single-cell suspensions were strained through nylon mesh. Each sample was analyzed using a FACSCalibur flow cytometer (BD Bioscience) using the FlowJo software (Tree Star, Ashland, OR).

2.5. Fluorescence imaging

Fluorescence images were taken using a BIOREVO BZ-9000 fluorescence microscope (KEYENCE, Osaka, Japan) immediately after each treatment. Time-lapse fluorescence and phase-contrast images were also taken using a BZ-9000 fluorescence microscope equipped with an incubation chamber held at 37 °C in a humidified atmosphere containing 5% CO_2 (Tokai Hit, Fujinomiya, Japan). Images were acquired every 10, 30, or 60 min.

2.6. Statistical analysis

Mean values were statistically compared using the two-tailed *t*-test. The association between abnormal fluorescence and mitotic catastrophe was examined by chi-square test. *P* values < 0.05 were considered statistically significant.

3. Results

3.1. Effects of plinabulin on cell-cycle distribution and mitosis in HeLa cells

The chemical structure of plinabulin is shown in Fig. 1A. Initially, we determined the effects of this compound on cell-cycle distribution in HeLa cells as a function of dose and exposure time. Flow-cytometric analysis revealed that significant G2/M arrest was observed at or above 10 nM; at 30 and 50 nM, most cells were arrested at G2/M phase after 24 h of treatment (Fig. 1Ba). All experiments described hereafter were performed at 50 nM. The G2/M arrest was apparent after 2 h of treatment, and gradually increased until almost all cells were arrested at G2/M after 16 h of treatment (Fig. 1Bb). To evaluate the effects of the agent on mitosis, we visualized chromosomes using GFP-tagged histone H2B. In the absence of plinabulin, we could clearly observe the separate stages of mitosis (prophase, metaphase, anaphase, and telophase), and the total duration was 55 ± 26 min ($n = 29$) (Fig. 1C, left panels). On the other hand, in the presence of plinabulin, the duration of mitosis

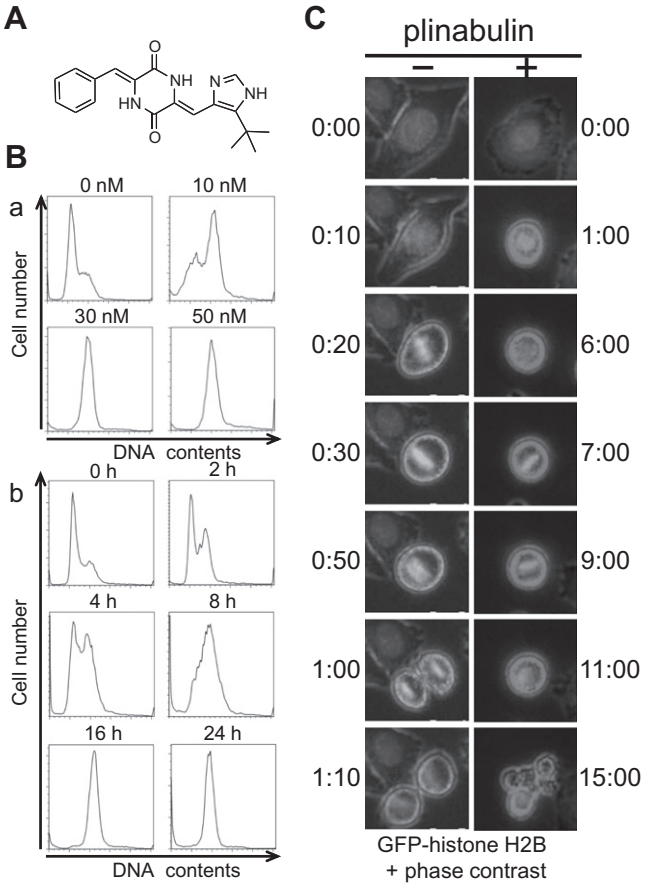

Fig. 1. Effects of plinabulin on cell-cycle distribution and mitosis. (A) Chemical structure of plinabulin. (B) Effects of plinabulin on cell-cycle distribution. (a) Dose response in cell-cycle distribution. After 24 h of treatment with plinabulin at various doses, cells were fixed and prepared for flow-cytometric analysis. (b) Time course of cell-cycle distribution. After treatment with 50 nM plinabulin for various times, cells were fixed and prepared for flow-cytometric analysis. (C) Typical examples of time-lapse imaging of HeLa-GFP-histone H2B after treatment with plinabulin. (−), Untreated cells; (+), cells treated with 50 nM plinabulin. The acquired time points are shown as hours:minutes in each image; 0:00 corresponds to late G2-phase prior to mitotic rounding.

was remarkably prolonged, and most cells collapsed during mitosis. The total duration of these abnormal mitoses, from prophase to collapse, was 9.4 ± 4.2 h (n = 50) (Fig. 1C, right panels). Although other microtubule-depolymerizing agents such as nocodazole were reported to induce cell-cycle arrest at prometaphase [13], in plinabulin-treated cells the chromosomes were aligned at the metaphase plate (Fig. 1C), albeit much later than in untreated cells. The metaphase-like phase continued for 1–7 h (3.9 ± 3.1 h) (n = 48); the plate began to expand, but collapsed within the mitosis (Fig. 1C, right panels). Collectively, these results imply that 50 nM plinabulin strongly inhibits cell-cycle progression at prometaphase or metaphase, and ultimately induces mitotic catastrophe.

3.2. The effects of plinabulin on fluorescence kinetics in HeLa-Fucci cells

To better understand the effects of plinabulin on the cell-cycle, we introduced the Fucci system [11] and attempted to visualize changes in cell-cycle progression in plinabulin-treated HeLa-Fucci cells. Representative fluorescence images for the indicated treatment times are shown in Fig. 2Aa. In untreated exponentially growing cells subjected to flow-cytometric analysis, the two-dimensional (2D) histogram of green and red fluorescence usually exhibits an inverse U-shaped pattern (Fig. 2Ab, 0 h). Fractions 1–4 represent early G1, G1, early S, and S/G2/M phases, respectively [11]. After plinabulin treatment, the histogram exhibited a gradual 'clockwise' progression and accumulation in Fr. 4′. Fucci probes are driven by a constitutively active CMV promoter [11], and expression increases when the cell cycle stops; accordingly, the population in Fr. 4 shifted to the right (Fig. 2Ab, 8 h and 16 h). A population not present in control cells emerged from the population with a higher mAG expression in Fr. 4′, and gradually progressed to Fr. 3′ (Fig. 2Ab, 8–24 h); ultimately, most cells accumulated in Fr. 3′ after 24 h of treatment (Fig. 2Ab, 24 h). Quantitative analysis of the newly appearing fraction is shown in Fig. 2Ac. Representative fluorescence images of HeLa-Fucci cells in the presence or absence of plinabulin are shown in Fig. 2B. Following mitotic changes in morphology, we observed that red fluorescence appeared in addition to the original green fluorescence; cells collapsed several hours after the appearance of the abnormal red fluorescence.

3.3. Close inspection of the timing of the appearance of the unusual red fluorescence

Next, we closely analyzed the time during the cell-cycle at which the unusual red fluorescence appeared. From the findings

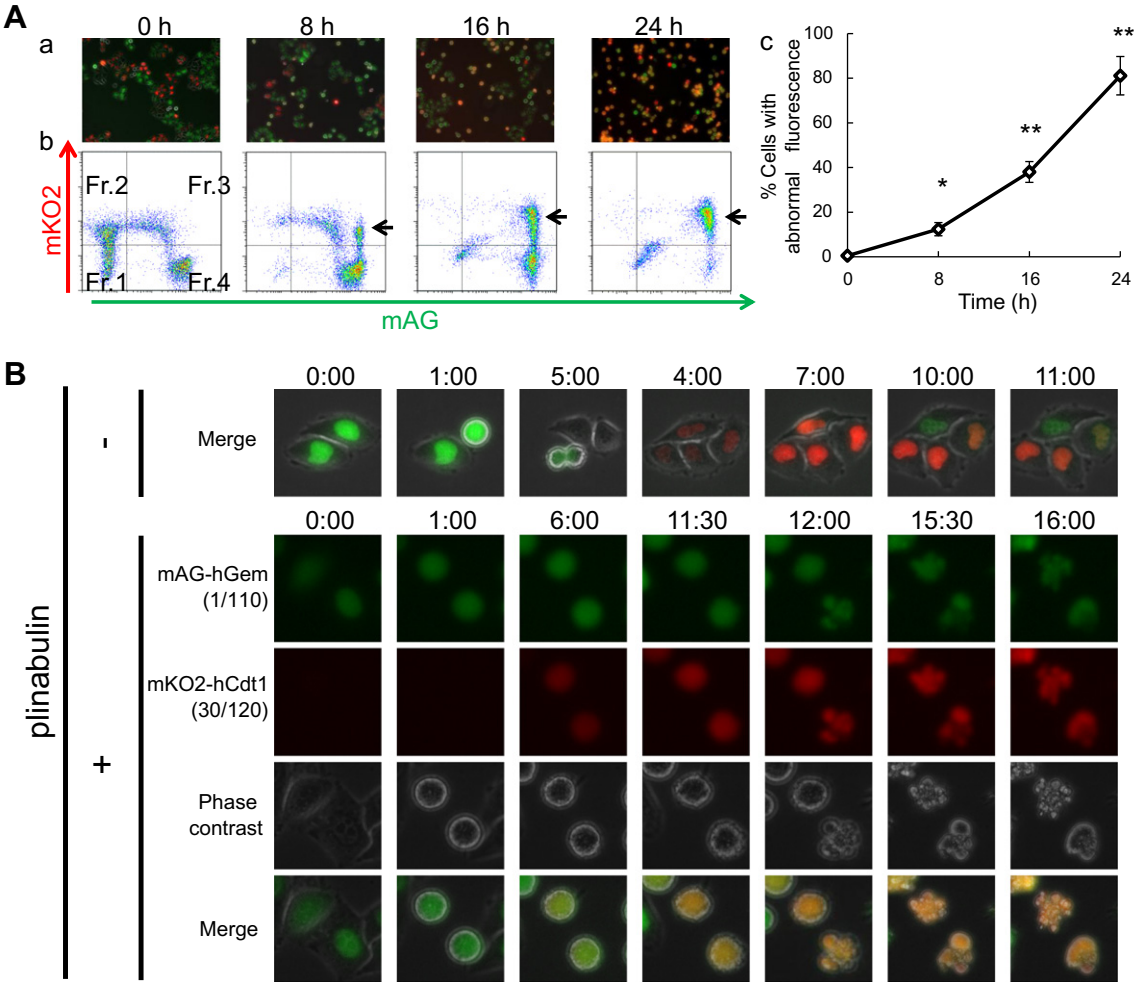

Fig. 2. Abnormal expression of red fluorescence during mitosis in HeLa-Fucci cells treated with plinabulin. (A) Fluorescence kinetics in HeLa-Fucci cells following plinabulin treatment. Representative fluorescence images (a) and 2D histograms of green and red fluorescence intensity obtained from flow-cytometric analysis (b) from HeLa-Fucci cells after treatment with 50 nM plinabulin for the indicated intervals. Arrows indicate abnormal fractions. (c) Quantitative analysis of abnormal fractions in plinabulin-treated cells, as observed in (b). Data presented are the mean +- SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. untreated cells. (B) Representative images of abnormal fluorescence patterns observed in plinabulin-treated HeLa-Fucci cells. The acquired time points are shown as hours:minutes in each image; 0:00 corresponds to late G2 phase prior to mitotic rounding.

described above, it was likely that the unusual pattern was appearing during mitosis. To confirm this, we combined X-irradiation with plinabulin treatment. Previously, we reported that X-irradiation induces almost complete arrest at the G2/M transition, lasting up to 20 h; this arrest is demonstrated by both fluorescence kinetics of the HeLa-Fucci and flow-cytometric analysis of DNA content [14]. When HeLa-Fucci cells were treated with plinabulin immediately after X-irradiation, many cells still accumulated in Fr. 4′ after 24 h of treatment; however, as the G2 arrest was released, the population in Fr. 3′ gradually increased (Fig. 3Aa–c). During that time, DNA content remained at 4 N (Fig. 3Ab, lower panels). These results indicate that irradiation inhibited cell-cycle progression at the G2/M transition, which retarded the unusual appearance of red fluorescence relative to the pattern observed after 24 h of treatment with plinabulin alone. Thus, we concluded that entrance into M phase is required for the unusual red fluorescence.

We next determined when during mitosis the unusual red fluorescence appeared. For this purpose, we transiently expressed the histone H2B-GFP fusion protein in HeLa-Fucci cells, in order to visualize changes in chromosome configuration, and performed time-lapse imaging of plinabulin-treated cells. A typical example is shown in Fig. 3B; the time at which the cell exhibited prophase was labeled as 0:00. At this stage, the Fucci mAG-Geminin probe containing the nuclear localization signal (NLS) was within the nucleus, and the red fluorescence did not appear. After an hour, we could identify nuclear envelope breakdown (NEBD) and clearly observe that the chromosomes were in a condensed configuration. At that time, the distribution of the probe had expanded to fill the entire cytoplasm, but the red fluorescence was still not detectable. Red fluorescence finally emerged about an hour before formation of a metaphase-like structure (Fig. 3B, 6 h and 7 h), and fluorescence intensity gradually increased (11 h). Another example is presented in Supplementary data.

3.4. Cell fate after the abnormal expression of red fluorescence

We next considered the significance of the emergence of the abnormal pattern. To further analyze the relationship between the pattern and cell collapse, we exposed cells to plinabulin for 24 h and then performed a pedigree assay in drug-free medium for an additional 48 h thereafter (Fig. 4A). Of randomly selected 96 cells that collapsed during this period of observation, 85 (88.5%) exhibited the abnormal pattern. On the other hand, in randomly selected 69 cells that did not collapse during this period of

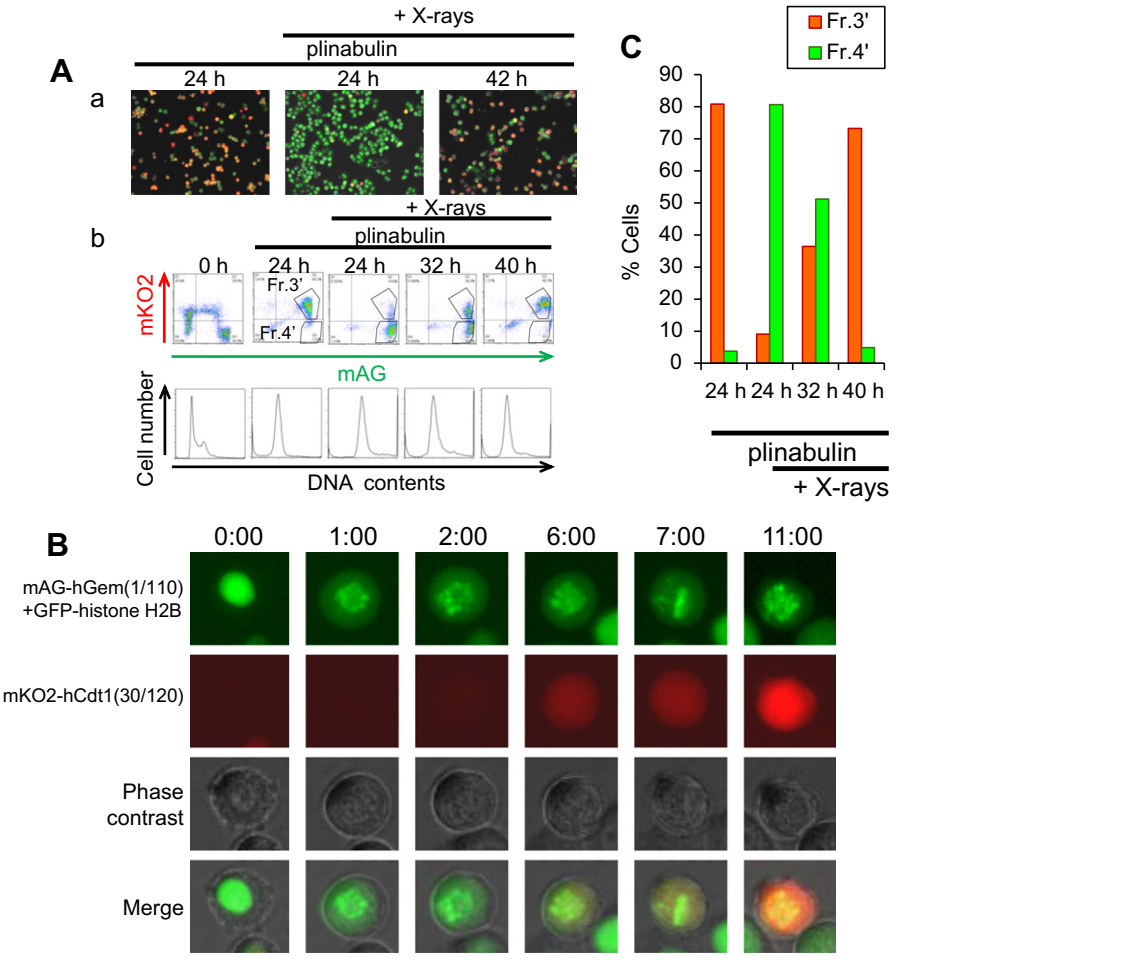

Fig. 3. Close inspection of the timing of emergence of the abnormal fluorescence in plinabulin-treated HeLa-Fucci cells. (A) Cell-cycle arrest at G2/M transition by X-irradiation retards expression of abnormal fluorescence. (a) Representative fluorescence images of plinabulin-treated cells, with or without X-irradiation. (b) 2D histograms of green and red fluorescence intensity (upper panels) and DNA content (lower panels) obtained by flow-cytometric analysis in plinabulin-treated cells, with or without X-irradiation. (c) Quantitative analysis of the abnormal fractions. Time course of Fr. 3′ and Fr. 4′ in (b) is shown. Cells were treated with 50 nM plinabulin immediately after X-irradiation at 8 Gy, and prepared for fluorescence observation or flow-cytometric analysis at the indicated times. (B) Typical examples of time-lapse images of plinabulin-treated HeLa-Fucci cells transiently expressing GFP-histone H2B. HeLa-Fucci cells were transduced with baculovirus containing GFP-histone H2B cDNA 16 h before plinabulin treatment and subsequently fluorescence images were acquired.

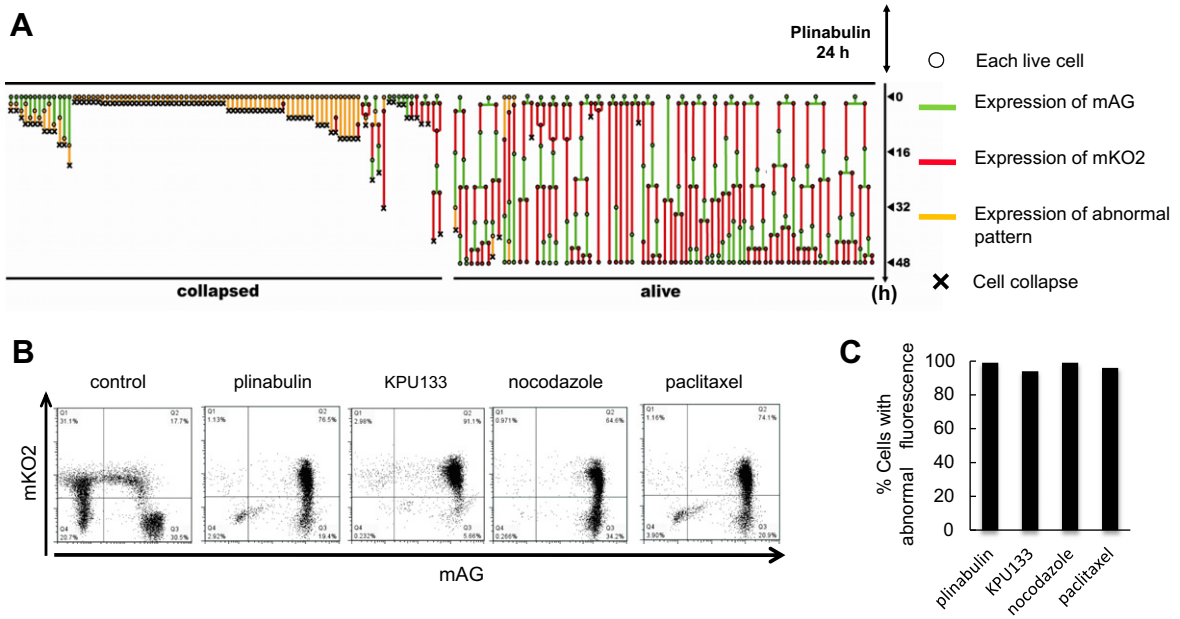

Fig. 4. Relationship between the abnormal fluorescence pattern and cell death. (A) Pedigree assay of HeLa-Fucci cells using time-lapse imaging. Fluorescence images were acquired in cells in fresh medium for 48 h following treatment with plinabulin for 24 h. At the end of the observation period, randomly selected 69 cells that were alive and 96 cells that had collapsed were analyzed. Cell division is represented by a horizontal line joining two circles; fusion of progenitors is represented by lines from two circles meeting at a single circle below them. Normal expression of both red and green fluorescence in early S phase is represented in red to avoid confusion with the abnormal fluorescence, which is depicted in orange. (B) Effects of other types of anti-microtubule agents on the induction of the abnormal fluorescence fractions. 2D histograms from flow-cytometric analysis after 24 h of each treatment. The doses of each agent were as follows: plinabulin, 50 nM; KPU133, 5 nM; nocodazole, 50 ng/mL; and paclitaxel, 50 nM. (C) Percentages of cells with abnormal red fluorescence in cells that collapsed after up to 48 h treatment. Time-lapse imaging analysis was performed during each treatment for 48 h. Each treatment dose was the same as in (B).

observation, only 3 (3.1%) exhibited the abnormal pattern. There was a statistically significant correlation between the emergence of the abnormal pattern and cell collapse (chi-square test, $p < 0.01$). We also investigated whether other types of microtubule-depolymerizing agents induce the abnormal pattern. We observed that KPU133 (a derivative of plinabulin with more potent cytotoxicity, 16j in Ref. [4]) and nocodazole both induced the unusual pattern (Fig. 4B). A microtubule-stabilizing agent, paclitaxel, also exerted a similar effect (Fig. 4B). Of the cells that ultimately collapsed after up to 48 h treatment with each anti-microtubule agent, more than 95% exhibited the unusual red fluorescence prior to cell death (Fig. 4C). These results suggest that the emergence of the abnormal red fluorescence is an early indicator of mitotic catastrophe.

4. Discussion

In our previous paper describing the characteristics of the Fucci system, we showed that nocodazole induced abnormal expression of red fluorescence, presumably in M phase [15]; however, at that time we did not investigate this phenomenon in detail. From a clinical viewpoint, this finding led us to characterize this phenomenon in detail using a novel anti-microtubule agent, plinabulin, which has yielded encouraging efficacy data in a phase 2 study [4]. The major novel findings in this study are as follows: (1) plinabulin inhibited the cell-cycle progression in M phase around prometaphase and metaphase; (2) this agent abnormally induced the red fluorescence after NEBD before the metaphase-like stage; (3) anti-microtubule agents including paclitaxel, a microtubule-stabilizing agent, also induced the phenomenon; and (4) the abnormal expression was followed by mitotic catastrophe.

The APC^{Cdh1} and SCF^{Skp2} complexes exhibit E3 ligase activities in a cell cycle-dependent manner and play key roles in cell-cycle progression; the former is active in late M and G1 phases, and the latter is active in S and G2 phases. The SCF^{Skp2} complex is a substrate of the APC^{Cdh1} complex and functions as a feedback inhibitor of APC^{Cdh1}. Therefore, the DNA replication licensing factor Cdt1 and its inhibitor Geminin, which are direct substrates of the SCF^{Skp2} complex and the APC^{Cdh1} complex, respectively, oscillate reciprocally during the cell cycle [11]. Thus, Cdt1 levels are augmented in G1, and Geminin levels are augmented in S, G2, and M phases [11,16]. Taking advantage of this cell cycle-specific ubiquitination, the Fucci system was developed by Sakaue-Sawano et al. as a tool to visualize cell cycle [11]. In this system, monomeric Azami Green (mAG)-hGem(1/110) probe (green) is expressed in G2, S, and M phases, while monomeric Kusabira Orange 2 (mKO2)-hCdt1(30/120) probe (red) is expressed in G1 phase [11]. In more detail, no fluorescence is emitted in early G1, whereas both red and green fluorescence signals are emitted in early S phase. We found that plinabulin abnormally induced red fluorescence in M phase, after NEBD and before a metaphase-like phase, ultimately leading to mitotic catastrophe. Based on the Fucci mechanism, red fluorescence should never be emitted at M phase during normal cell-cycle progression. In order to allow expression of mKO2-hCdt1(30/120) when the APC^{Cdh1} complex is not activated, SCF^{Skp2} complex activity must somehow be inactivated at early M phase following treatment with anti-microtubule agents. Further study will be required to elucidate how this dysregulation of E3 ligase occurs, and whether it is linked to mitotic catastrophe.

Hashimoto et al. reported a very similar phenomenon in M phase-arrested HeLa-Fucci cells following treatment with caspase inhibitors [17]. Those authors claim that caspase-7 activity, which is independent of apoptotic signals, is normally required for the transition from M to G1 phase. According to their view, caspase inhibition blocks cell-cycle progression at M phase. Anti-microtubule agents also induce cell cycle arrest at M phase, and may therefore induce the abnormal fluorescence by a similar mechanism. This cell-cycle arrest may impair the regulation of APC activity at the transition from M to G1 phase. Neither the molecular

mechanism of the emergence of the red fluorescence nor any possible association of cell death was explored in that study. The findings we report here hint at a strategy for enhancing anti-tumor effects by anti-microtubule agents; therefore, it will be important to elucidate the molecular mechanisms underlying the phenomenon.

Acknowledgments

The authors thank Dr. A. Miyawaki and Dr. A. Sakaue-Sawano for providing the HeLa cells expressing the Fucci probes. We also thank Dr. H. Saya for providing HeLa cells stably expressing GFP-histone H2B. This study was supported in part by a Grant-in-Aid for Scientific Research from MEXT, Japan (23390427) (to M.M.).

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.2012.10.014.

References

[1] E. Nogales, Structural insights into microtubule function, Annu. Rev. Biochem. 69 (2000) 277–302.
[2] J. Zhou, P. Giannakakou, Targeting microtubules for cancer chemotherapy, Curr. Med. Chem. Anti-Cancer Agents 5 (2005) 65–71.
[3] C. Dumontet, M.A. Jordan, Microtubule-binding agents: a dynamic field of cancer therapeutics, Nat. Rev. Drug Discov. 9 (2010) 790–803.
[4] Y. Yamazaki, M. Sumikura, Y. Masuda, Y. Hayashi, H. Yasui, Y. Kiso, T. Chinen, T. Usui, F. Yakushiji, B. Potts, S. Neuteboom, M. Palladino, G.K. Lloyd, Y. Hayashi, Synthesis and structure-activity relationships of benzophenone-bearing diketopiperazine-type anti-microtubule agents, Bioorg. Med. Chem. 20 (2012) 4279–4289.
[5] C.M. Lin, H.H. Ho, G.R. Pettit, E. Hamel, Antimitotic natural products combretastatin A-4 and combretastatin A-2: studies on the mechanism of their inhibition of the binding of colchicine to tubulin, Biochemistry 28 (1989) 6984–6991.
[6] L.G. Daenen, J.M. Roodhart, Y. Shaked, E.E. Voest, Vascular disrupting agents (VDAs) in anticamcer therapy, Curr. Clin. Pharmacol. 5 (2010) 178–185.
[7] B. Nicholson, G.K. Lloyd, B.R. Miller, M.A. Palladino, Y. Kiso, Y. Hayashi, S.T.C. Neulteboom, NPI-2358 is a tubulin-depolymerizing agent: in-vitro evidence for activity as a tumor vascular-disrupting agent, Anti-Cancer Drugs 17 (2006) 25–31.
[8] Y. Yamazaki, K. Tanaka, B. Nicholson, G. Deyanat-Yazdi, B. Potts, T. Yoshida, A. Oda, T. Kitagawa, S. Orikasa, Y. Kiso, H. Yasui, M. Akamatsu, T. Chinen, T. Usui, Y. Shinozaki, F. Yakushiji, B.R. Miller, S. Neuteboom, M. Palladino, K. Kanoh, G.K. Lloyd, Y. Hayashi, Synthesis and structure-activity relationship study of antimicrotubule agents phenylahistin derivatives with a didehydropiperazine-2,5-dione structure, J. Med. Chem. 55 (2012) 1056–1071.
[9] M.M. Mita, M.A. Spear, L.K. Yee, A.C. Mita, E.I. Heath, K.P. Papadopoulos, K.C. Federico, S.D. Reich, O. Romero, L. Malburg, M. Pilat, G.K. Lloyd, S.T. Neuteboom, G. Cropp, E. Ashton, P.M. LoRusso, Phase 1 first-in-human trial of the vascular disrupting agent plinabulin (NPI-2358) in patients with solid tumors or lymphomas, Clin. Cancer Res. 16 (2010) 5892–5899.
[10] A.V. Singh, M. Bandi, N. Raje, P. Richardson, M.A. Palladino, D. Chauhan, K.C. Anderson, A novel vascular disrupting agent plinabulin triggers JNK-mediated apoptosis and inhibits angiogenesis in multiple myeloma cells, Blood 117 (2011) 5692–5700.
[11] A. Sakaue-Sawano, H. Kurokawa, T. Morimura, A. Hanyu, H. Hama, H. Osawa, S. Kashiwagi, K. Fukami, T. Miyata, H. Miyoshi, T. Imamura, M. Ogawa, H. Masai, A. Miyawaki, Visualizing spatiotemporal dynamics of multicellular cell-cycle progression, Cell 132 (2008) 487–498.
[12] M. Nitta, O. Kobayashi, S. Honda, T. Hirota, S. Kuninaka, T. Marumoto, Y. Ushio, H. Saya, Spindle checkpoint function is required for mitotic catastrophe induced by DNA-damaging agents, Oncogene 23 (2004) 6548–6558.
[13] A.L. Blajeski, V.A. Phan, T.J. Kottke, S.H. Kaufmann, G1 and G2 cell-cycle arrest following microtubule depolymerization in human breast cancer cells, J. Clin. Invest. 110 (2002) 91–99.
[14] A. Kaida, M. Miura, Visualizing the effect of hypoxia on fluorescence kinetics in living HeLa cells using the fluorescent ubiquitination-based cell cycle indicator (Fucci), Exp. Cell Res. 318 (2012) 288–297.
[15] A. Kaida, N. Sawai, K. Sakaguchi, M. Miura, Fluorescence kinetics in HeLa cells after treatment with cell cycle arrest inducers visualized with Fucci (fluoresent ubiquitination-based cell cycle indicator), Cell Biol. Int. 35 (2011) 359–363.
[16] H. Nishitani, Z. Lygerou, T. Nishimoto, Proteolysis of DNA replication licensing factor Cdt1 in S-phase is performed independently of geminin through its N-terminal region, J. Biol. Chem. 279 (2004) 30807–30816.
[17] T. Hashimoto, U. Kikkawa, S. Kamada, Contribution of caspase(s) to the cell cycle regulation at mitotic phase, Plos ONE 6 (2011) e18449.