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Real-Time Fluorescence Imaging of the DNA Damage Repair Response During Mitosis

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ABSTRACT

The response to DNA damage during mitosis was visualized using real-time fluorescence imaging of focus formation by the DNA-damage repair (DDR) response protein 53BP1 linked to green fluorescent protein (GFP) (53BP1-GFP) in the MiaPaCa-2$^{Tet\text{-}On}$ pancreatic cancer cell line. To observe 53BP1-GFP foci during mitosis, MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells were imaged every 30 min by confocal microscopy. Time-lapse imaging demonstrated that 11.4 ± 2.1% of the mitotic MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells had increased focus formation over time. Non-mitotic cells did not have an increase in 53BP1-GFP focus formation over time. Some of the mitotic MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells with focus formation became apoptotic. The results of the present report suggest that DNA strand breaks occur during mitosis and undergo repair, which may cause some of the mitotic cells to enter apoptosis in a phenomenon possibly related to mitotic catastrophe. J. Cell. Biochem. 116: 661–666, 2015.

KEY WORDS: GFP; 53BP1; FUSION GENE; DNA DAMAGE; DNA REPAIR RESPONSE; FOCUS FORMATION; MITOSIS; MITOTIC CATASTROPHE; APOPTOSIS; TIME-LAPSE CONFOCAL IMAGING

Efimova et al. [2010] fused green fluorescent protein (GFP) and the DNA damage-response (DDR) protein 53BP1 for use as a live-cell imaging reporter for DNA repair.

In our previous studies on the effect of ultraviolet (UV) light on cancer cells, we have shown various types of cancer are readily killed by UVC in vitro and in vivo and that expression of fluorescent proteins by the cancer cells enhances their killing by UVC [Kimura et al., 2010; Tsai et al., 2010; Momiyama et al., 2012; Hoffman and Bouvet, 2012; Miwa et al., 2013a, b; Momiyama et al., 2013; Hiroshima et al., 2014; Uehara et al., 2014].

Subsequently, we have shown that UVC-induced DNA repair was reported by focus formation by 53BP1-GFP in MiaPaCa-2 human pancreatic cancer cells in monolayer culture [Miwa et al., 2013a].

UVC induction of DNA damage repair was also observed by 53BP1-GFP focus formation in MiaPaCa-2 cells growing in 3-dimensional Gelfoam® histoculture [Miwa et al., 2013b].

In vivo experiments demonstrated that UVB as well as UVC could induce 53BP1-GFP focus formation in MiaPaCa-2 cells which were seeded in skin flaps in nude mice. UVB penetrated the growing MiaPaCa-53BP1 tumor to greater depths than UVC as indicated by 53BP1 focus formation and had greater inhibitory efficacy against the tumor [Uehara et al., 2014].

The present report demonstrates that the DNA-damage repair response occurs during mitosis of MiaPaCa-2$^{Tet\text{-}On}$ cells as observed by 53BP1-GFP focus formation and that some of focus-forming mitotic cells enter apoptosis.

This paper is dedicated to the memory of A. R. Moossa, M.D.

Conflict of interest: None of the authors have a conflict of interest with regard to this study.

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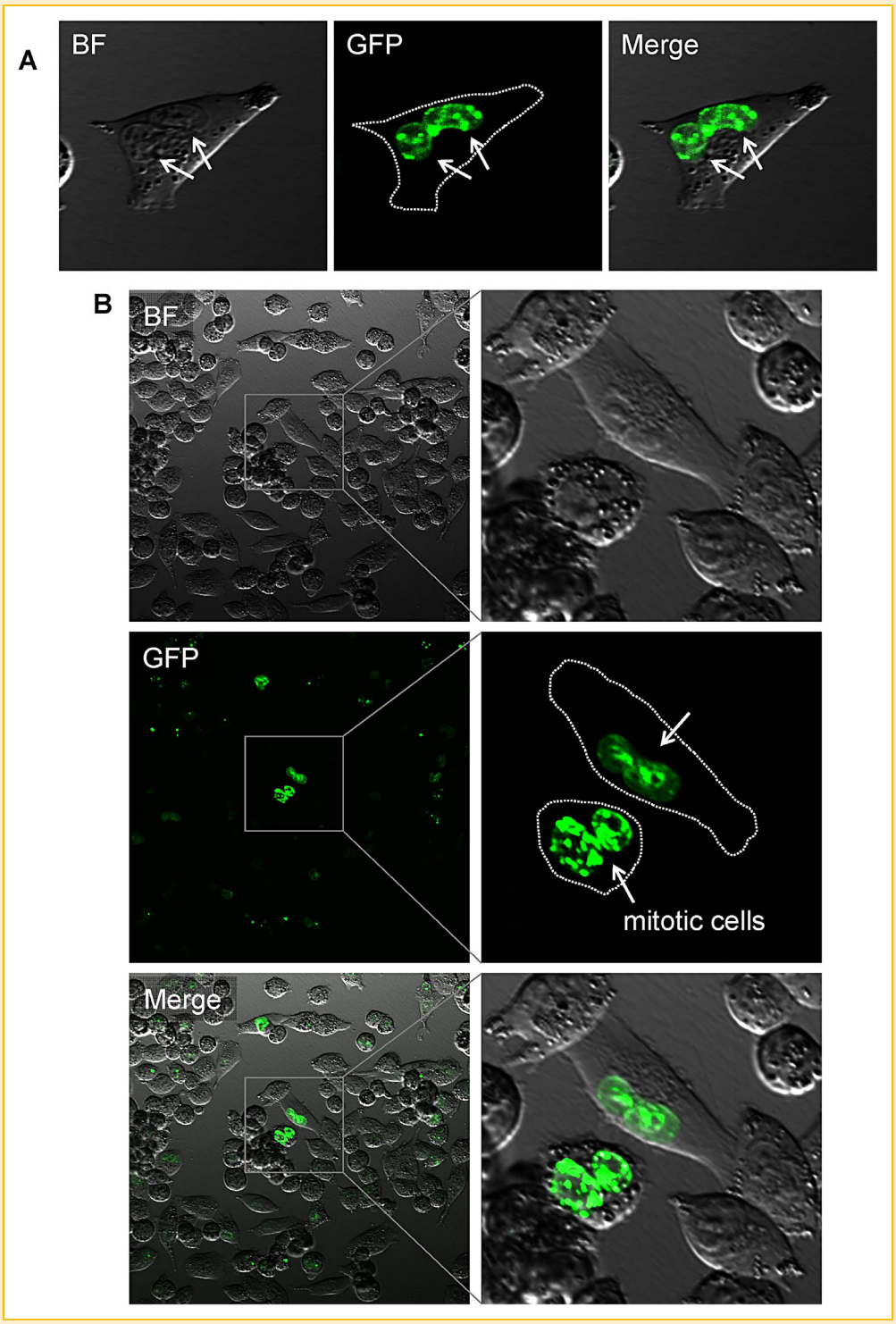

Fig. 1. Focus formation in MiaPaCa-2^{Tet-On} 53BP1-GFP cells. MiaPaCa-2^{Tet-On} 53BP1-GFP cells were seeded in 35 mm dishes and treated with 1 μg/ml doxycycline for 48 h. A: 53BP1-focus formation was observed with the FV1000 confocal microscope. B: Many of the focus-positive cells were observed undergoing mitosis. 53BP1-GFP foci are indicated by arrows. BF = brightfield.

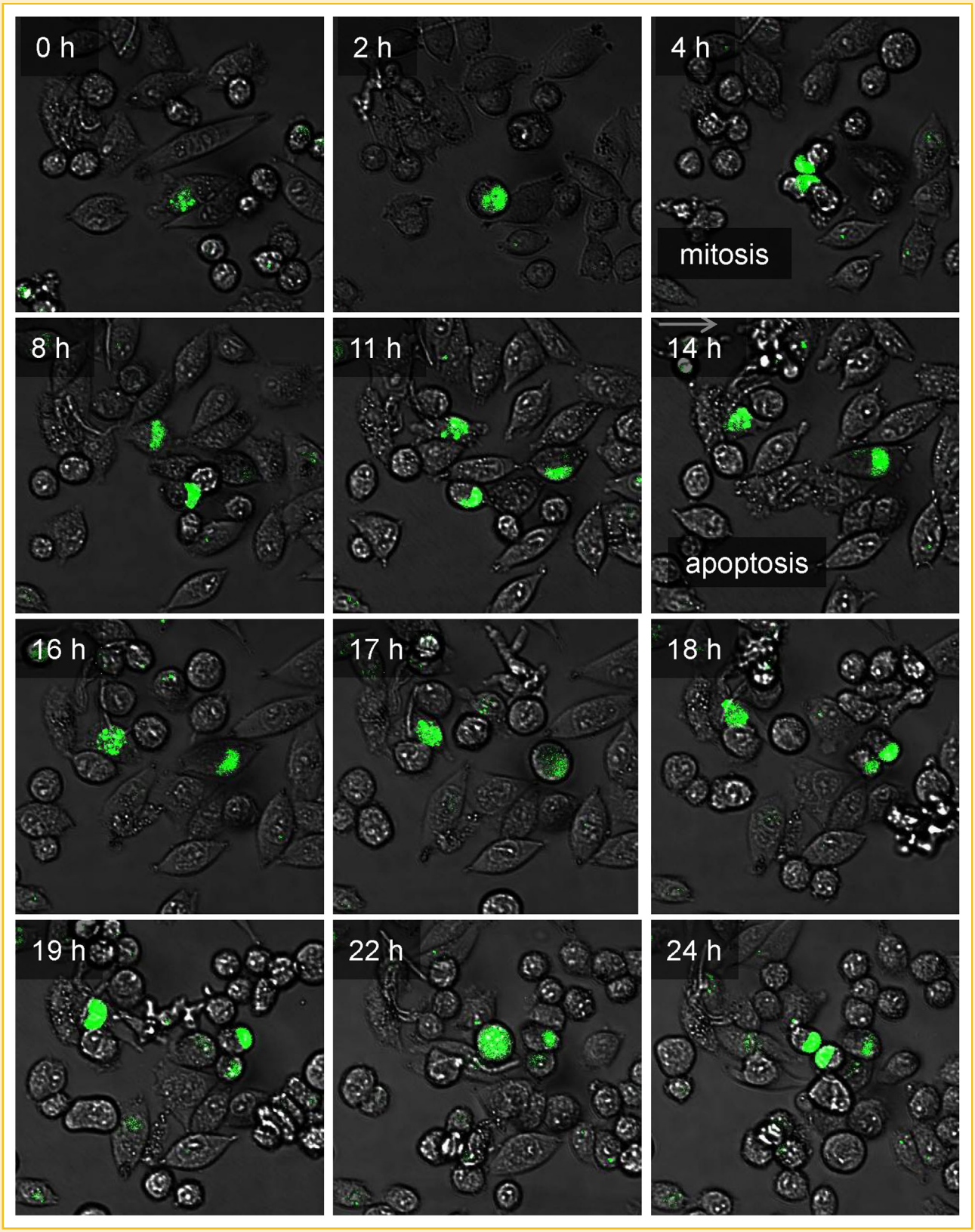

Fig. 2. Time-lapse imaging of 53BP1-GFP focus formation during mitosis. MiaPaCa-2^{Tet-On} 53BP1-GFP cells were seeded in 35 mm dishes and treated with 1 μg/ml doxycycline for 48 h. 53BP1-GFP focus formation was observed every 30 min for 24 h with the FV1000 confocal microscope (Supplementary movie 1). Time-lapse imaging demonstrated that many cells formed 53BP1-GFP foci during mitosis. Focus formation persisted after mitosis. Some focus-positive cells entered apoptosis.

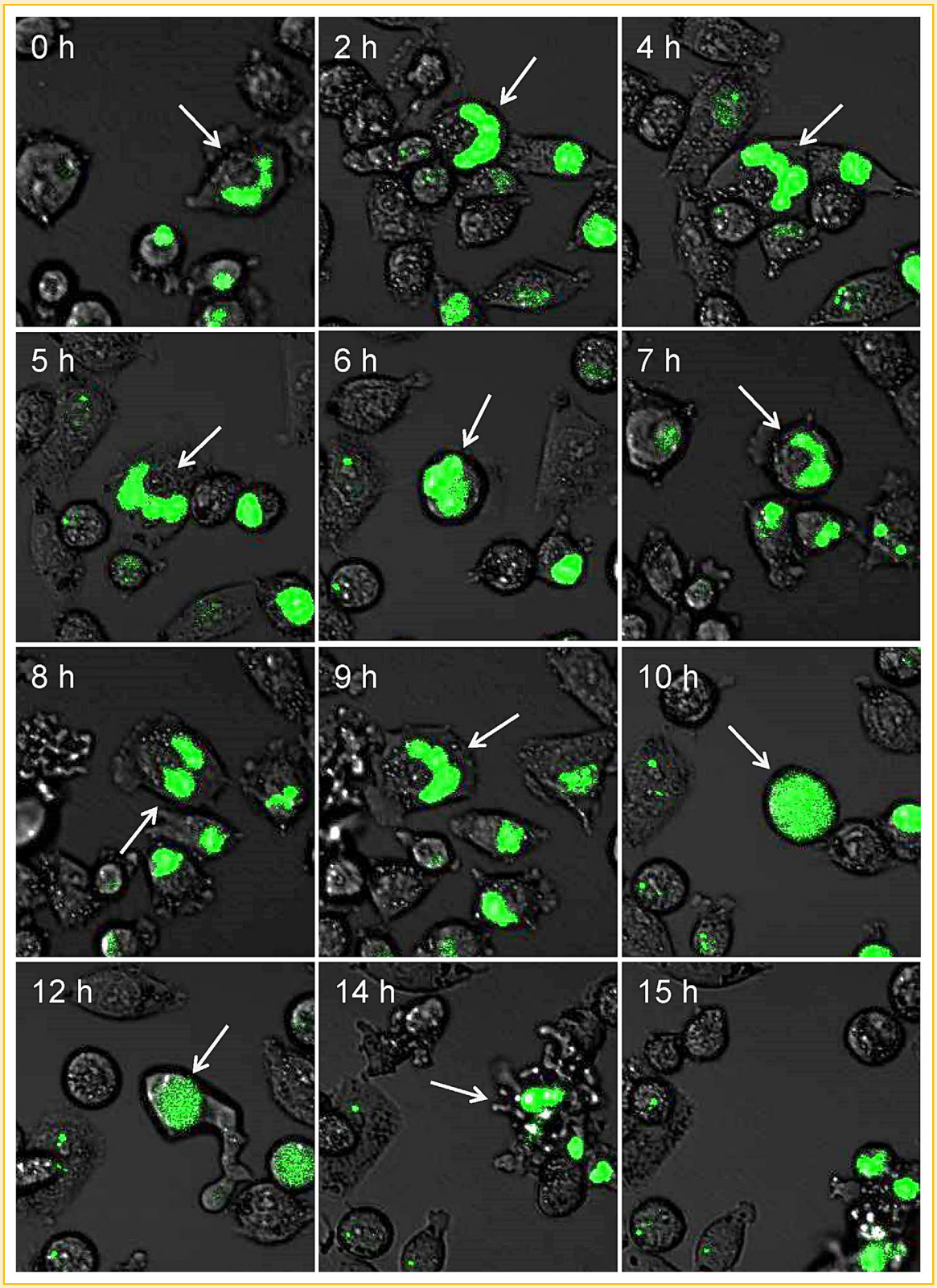

Fig. 3. Time-lapse imaging of mitotic reversal and induction of apoptosis in 53BP1-GFP focus-forming cells. MiaPaCa-2^{Tet-On} 53BP1-GFP cells were seeded in 35 mm dishes and treated with 1 μg/ml doxycycline for 48 h. 53BP1-GFP focus formation was observed every 30 min for 24 h with the FV1000 confocal microscope (Supplementary movie 2). Time-lapse imaging demonstrated that 53BP1-GFP focus-positive cells (arrows) had nuclear division (7–8 h). The divided nuclei appeared to re-fuse (8–10 h), and then entered apoptosis (12–15 h).

MATERIALS AND METHODS

CELL CULTURES AND CONSTRUCTS

The MiaPaCa-2^{Tet-On} Advanced cells line (Clontech, Carlsbad, CA) was transfected by 53BP1 fused to GFP in a lentiviral vector (Clontech) [Efimova et al., 2010; Miwa et al., 2013a, b, c]. MiaPaCa-2 53BP1-GFP cells were cultured in DMEM medium containing high glucose (Invitrogen, Carlsbad, CA) using fetal bovine serum (10%) approved for Tet systems [Efimova et al., 2010; Miwa et al., 2013a, b, c].

FLUORESCENCE IMAGING OF 53BP1 FOCUS FORMATION DURING MITOSIS

To observe the response to DNA damage during mitosis, MiaPaCa-2^{Tet-On} 53BP1-GFP cells were seeded on 35 mm dishes, treated with 1 μg/ml doxycycline for 48 h and observed every 30 min with the FV1000 confocal laser microscope (Olympus Corp., Tokyo, Japan). Images were captured on a computer and Cell® software (Olympus Biosystems) was used for image analysis. The cells which contained five or more foci were considered as positive cells. The experimental data are expressed as the mean ± SE [Miwa et al., 2013a, b, c; Uehara et al., 2014].

RESULTS AND DISCUSSION

FOCUS FORMATION DURING MITOSIS OF MiaPaCa-2^{TET-ON} 53BP1-GFP CELLS

To observe response to DNA damage in mitotic cancer cells, MiaPaCa-2^{Tet-On} 53BP1-GFP cells were treated with 1 μg/ml doxycycline for 48 h. 53BP1-GFP foci were observed with the FV1000 confocal microscope. For MiaPaCa-2^{Tet-On} 53BP1-GFP, 2.8 ± 0.8% of the cells were focus positive, and 81.5 ± 11.3% of the focus-positive cells entered mitosis. Focus formation of 53BP1-GFP in mitotic cells indicated that response to DNA damage frequently occurs during mitosis (Fig. 1).

TIME-LAPSE IMAGING OF FOCUS FORMATION DURING MITOSIS IN MiaPaCa-2^{TET-ON} 53BP1-GFP CELLS

To visualize the time course of DNA damage response in mitotic cancer cells, MiaPaCa-2^{Tet-On} 53BP1-GFP cells were first treated with 1 μg/ml doxycycline for 48 h. 53BP1-GFP focus formation was observed every 30 min with the FV1000 confocal microscope for 24 h. Time-lapse single-cell confocal imaging of MiaPaCa-2^{Tet-On} 53BP1-GFP cells demonstrated that 53BP1 foci increased in number during mitosis (Fig. 2, Supplementary Video 1), and the increased foci persistsed after mitosis. The increased expression of 53BP1-GFP during mitosis indicates that DNA repair is needed during replication of DNA. Furthermore, time-lapse imaging also demonstrated that after the appearance of 53BP1-GFP foci during mitosis, the cells frequently entered apoptosis. During 24 h time-lapse imaging, mitosis was observed 1.1 ± 0.1 times in each cell, and apoptotic changes were observed in 7.3 ± 1.9% of the mitotic cells. 53BP1-GFP focus formation was observed in 11.4 ± 2.1% of the mitotic cells during the 24 h observation period.

RE-FUSION OF 53BP1-GFP EXPRESSING NUCLEI AFTER MITOSIS

Time-lapse imaging of 53BP1-GFP focus formation demonstrated division of 53BP1 focus-positive nuclei and subsequent re-fusion of some of the divided nuclei, and subsequent induction of apoptosis in some of the cells (Fig. 3, Supplementary Video 2). This phenomenon indicates that response to DNA damage is activated during mitosis, and that DNA damage may lead to an apparent "mitotic reversal" and subsequent apoptosis. DNA damage signaling from unrepairable lesions may activate apoptosis or trigger cell cycle "mitotic reversal" in order to prevent inheritance of aberrant DNA [Giunta and Jackson, 2011].

In this study, the response to DNA damage during mitosis was visualized using fluorescent protein-based real-time imaging of 53BP1-GFP focus formation in mitotic MiaPaCa-2^{Tet-On} human pancreatic cancer cells. Time-lapse imaging demonstrated that 11.4 ± 2.1% of the mitotic cells formed 53BP1-GFP foci. Non-mitotic cells did not have an increase in 53BP1-GFP focus formation over time. Time-lapse imaging also showed that some of the mitotic MiaPaCa-2^{Tet-On} 53BP1-GFP cells with increased focus formation became apoptotic. The results of the present report suggest that DNA strand breaks occur during mitosis and undergo repair, and if the repair is insufficient, apoptosis results.

Our previous results demonstrated that DNA damage repair by UVC irradiation can be imaged by 53BP1-GFP focus formation in cancer cells [Miwa et al., 2013a] including in three-dimensional Gelfoam® histoculture which is an in vivo-like culture system [Hoffman 2013; Tome et al., 2014]. Our previous results also demonstrated that 53BP1-GFP focus formation could be imaged in minimal cancer in vivo [Miwa et al., 2013b]. MiaPaCa-2^{Tet-On}-53BP1 GFP cells irradiated by UVB or UVC in the skin-flap mouse model had a significant increase in focus formation, with UVB having more focus induction than UVC, possibly due to greater penetration by UVB [Uehara et al., 2014].

The present study demonstrated the DNA damage repair response occurs spontaneously in mitotic cells.

Mitotic catastrophe, where cells die during or after mitosis, may occur as a result of mis-repair of DNA damage [Castedo et al., 2004; Vakifahmetoglu et al., 2008; Chow and Poon, 2010], which may be what we have observed in the present report. Future experiments will image the fate of B3BP1-GFP focus-forming mitotic cells which did not undergo apoptosis, to determine if they have become more malignant.

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