Efficacy of the Combination of a PARP Inhibitor and UVC on Cancer Cells as Imaged by Focus Formation by the DNA Repair-related Protein 53BP1 Linked to Green Fluorescent Protein

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Abstract. *Background: The ability to image DNA repair in cancer cells after irradiation, as well as its inhibition by potential therapeutic agents, is important for the further development of effective cancer therapy. 53BP1 is a DNA repair protein that is overexpressed and forms foci when double-stranded DNA breaks occur in DNA. Materials and Methods: The re-localization of green fluorescent protein (GFP) fused to the chromatin-binding domain of 53BP1 to form foci was imaged after UVC irradiation of breast and pancreatic cancer cells expressing 53BP1-GFP using confocal microscopy. Results: During live-cell imaging, 53BP1-GFP focus formation was observed within 10 minutes after UVC irradiation. Most 53BP1 foci resolved by 100 minutes. To block UVC-induced double-strand break repair in cancer cells, poly(ADP-ribose) polymerase (PARP) was targeted with ABT-888 (veliparib). PARP inhibition markedly enhanced UVC-irradiation-induced persistence of 53BP1-foci, even beyond 100 minutes after UVC irradiation, and reduced proliferation of breast and pancreatic cancer cells. Conclusion: Confocal microscopy of 53BP1-GFP is a powerful method for imaging UVC-induced DNA damage and repair, as well as inhibition of repair.*

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Our laboratory pioneered the use of green fluorescent protein (GFP) for *in vivo* imaging in 1997 (1, 2). With the use of GFP, it became possible to observe individual cancer cells in fresh unstained tissue or even a live animal for the first time. Fluorescent proteins can be used to visualize primary tumor growth, tumor cell motility and invasion, metastatic seeding and colonization, angiogenesis, as well as the interaction between the tumor and its microenvironment (3-8).

Photodynamic therapy has been shown to be effective for certain cancer types (9). Recently, blue light was found to be phototoxic for both murine and human melanoma (10). UV light has been used for the phototherapy of cutaneous malignancies. Psoralen plus UVA (PUVA) and narrowband UVB were the most common phototherapy modalities utilized (11-13). However, the effect of UV light on cancer cells is not well understood (14-16). Our previous study demonstrated that UVC causes cancer cell death *in vitro* and *in vivo* (17).

Small molecules targeting cellular response to DNA damage are potential cancer therapeutics (18). The double-strand break response involves rapid recruitment and activation of poly(ADP-ribose) polymerase 1 (PARP1) (19-21). PARP inhibitors can act as sensitizers for DNA-damaging agents for cancer therapy (22-26).

We previously demonstrated that UV-induced DNA damage and repair can be visualized by imaging focus formation of GFP fused to the chromatin-binding domain of 53BP1 (27-29). In the present study, we used this imaging strategy to observe

the efficacy of the PARP inhibitor, ABT-888 {veliparib; 2-[(*R*)-2-methyl-pyrrolidin-2-yl]-1*H*-benzimidazole-4-carboxamide}, on blockage of 53BP1-GFP focus resolution and proliferation of breast cancer and pancreatic cancer cell lines. These results suggests UVC-irradiation and PARP inhibition are a promising therapeutic combination for breast and pancreatic cancer.

Materials and Methods

Cell lines and culture. MiaPaCa-2$^{Tet\text{-}On}$-53BP1-GFP and MCF7^{TetOn}-53BP1-GFP cells (MiaPaCa-2-53BP1-GFP and MCF7-53BP1-GFP, respectively) were certified by Clontech (Mountain View, CA, USA) or other manufacturers. Cells were maintained in high-glucose DMEM with 10% Tet system-approved fetal bovine serum (Clontech), G418 (200 μg/ml), and puromycin (0.5 μg/ml), after induction of 53BP1 expression for 48 hours with doxycycline (1 μg/ml) (Sigma, St. Louis, MO, USA).

UVC irradiation. For UV irradiation, cells were cultured in 6-well plates. The cells were irradiated from the top using a Benchtop 3UV transilluminator (UVP LLC, Upland, CA, USA) or conventional culture hood UV light. The UV dose was measured with a UVX Radiometer (UVP) or equivalent (17).

UV-induced cancer cell death. In order to determine if UV-induced cancer cell death was dose-dependent, MCF7-53BP1-GFP and MiaPaCa-2-53BP1-GFP were seeded in 6-well plates. After 48 h culture, cells were irradiated with different doses of UVC (25-100 J/m^2). Cells were pretreated with 10 μmol/l ABT-888 before UVC irradiation. Ten days after irradiation with or without ABT-888 (Santa Cruz Biotech, Santa Cruz, CA, USA) treatment, the number of cancer colonies were determined by a clonogeneic assay with replicates and performed twice independently.

Clonogenic assay. Clonogenic assays were performed with crystal violet staining according to standard protocols (30).

Live-cell 53BP1 imaging in vitro and quantification of focus number with or without UV treatment in the presence or absence of ABT-888. Live-cell images were captured with an Olympus Fluoview 1000 laser scanning confocal microscope equipped with a XLUMPLFL 20× (0.95 NA) water-immersion objective (31). GFP was excited with a 488 nm laser line of an argon ion laser. Image stacks were obtained through entire nuclei in 8 random fields of cells using 0.33 μm z-steps.

Results and Discussion

53BP1-GFP reports UVC DNA damage and repair in living cells. Following induction with doxycycline, unirradiated MCF7-53BP1-GFP cells (Figure 1A and 1B) contained only rare nuclear foci (mean=1.2 ± 2.3 per cell), consistent with a previous report (24). Unirradiated MiaPaCa-2-53BP1-GFP cells (Figure 2A) also contained only rare nuclear foci (mean=1.3 ± 2.3 per cell after induction with deoxycycline) (Figure 2A and 2B). The 53BP1-GFP re-localized within minutes after UVC irradiation (25 J) to form nuclear foci, thereby reporting DNA damage. The 53BP1-GFP foci then resolved relatively quickly over the next 90 minutes, thereby reporting DNA repair.

In both MCF7-53BP1-GFP and MiaPaCa-2-53BP1-GFP cells, 53BP1 foci increased after UVC irradiation (Figure 1B and 2B). After 5 minutes, MCF7-53BP1-GFP cells had a mean ± SD of 29.8 ± 11.0 53BP1 foci and MiaPaCa-2 cells had a mean of 32.0 ± 11.0. After 10 minutes, the number of foci rose to 32.5 ± 9.7 and 34.2 ± 9.2, respectively. After 15 minutes, MCF7-53BP1-GFP cells had a mean of 33.8 ± 9.9 and MiaPaCa-2 cells a mean of 37.3 ± 8.9 53BP1 foci.

UVC-induced cancer cell death. After exposure to different doses of UVC, the number of cancer colonies was quantitated with a clonogenic assay (Figures 3A and 4A). As little as 25 J/m^2 UVC irradiation killed approximately 65% of both MCF7-53BP1-GFP and MiaPaCa-2-53BP1-GFP cells. This is consistent with 53BP1-GFP focus formation at 25 J UVC irradiation. The frequency of cell killing plateaued at 75 J/m^2 (Figures 3B and 4B).

PARP 1 inhibitor ABT-888 caused persistence of 53BP1-GFP foci, suppressing cell proliferation. Treating MCF7-53BP1-GFP and MiaPaCa-2-53BP1-GFP cells with UVC irradiation in the presence of the PARP 1 inhibitor ABT-888 (24, 30, 32) markedly increased 53BPI-GFP focus formation (Figures 5A and 6A). Time-lapse live-cell imaging of 53BP1-GFP demonstrated that in cells treated with UVC only, 53BP1-GFP appeared within 10 minutes and began to decrease noticeably by 70 minutes (Figures 5B and 6B). However, after combined treatment with UVC plus ABT-888, 53BP1-GFP foci persisted for greater than 70 minutes. Treatment with ABT-888 alone slightly reduced colony formation at 10 μmol/l (MCF7-53BP1-GFP: 95.1 ± 6.1% of control, MiaPaCa-2-53BP1-GFP; 92.6 ± 9.8% of control). However, ABT-888 significantly reduced colony formation following 25 J/m^2 (UVC alone *versus* UVC + ABT-888 relative to the control: 34.1 ± 1.1% *versus* 8.7 ± 1.9%, respectively, in MCF7-53BP1-GFP cells; 31.8 ± 2.8% *versus* 12.4 ± 3.1%, respectively, in MiaPaCa-2-53BP1-GFP; $p<0.01$ Student *t*-test, respectively), with similar fold reductions at UVC doses up to 50 J/m^2 (Figures 5C and 6C).

Our results indicated that UVC light induces cell death in both breast and pancreatic cancer cells, consistent with our previous report (17). Furthermore, our results demonstrate enhancement of UVC irradiation efficacy by PARP inhibition and further implicate UVC irradiation-induced foci as a potential indicator of DNA damage. The results in the present report indicate that the efficacy of UVC on cancer cell death is time- and dose-dependent. Although UVC light does not deeply penetrate tissue, we assumed that a combination of UVC irradiation with PARP inhibition could be more effective to accelerate tumor cell death in clinical settings, at least with superficial cancer, including minimal residual disease (17).

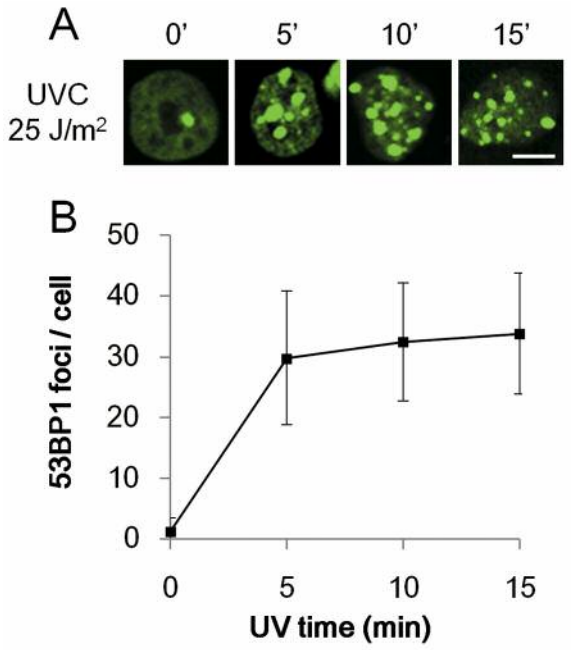

Figure 1. *Kinetics of 53BP1-GFP focus formation after UVC irradiation 0f MCF7-53BP1-GFP human breast cancer cells. A: Formation of 53BP1-GFP foci in response to UVC (25 J/m^2) with respect to duration of irradiation. Bar=10 μm. B: Average number of 53BP1-GFP foci per cell plotted against total time of irradiation (n >50) UVC irradiation (25 J/m^2). Bars=SD.*

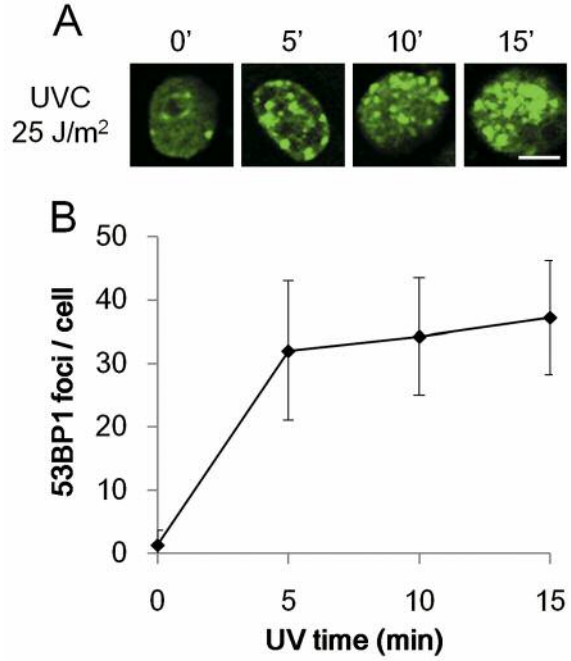

Figure 2. *Kinetics of 53BP1-GFP focus formation after UVC irradiation of MiaPaCa-2-53BP1-GFP human pancreatic cancer cells. A: Formation of 53BP1-GFP foci in response to UVC with respect to duration of irradiation. Bar=10 μm. B: Average number of 53BP1-GFP foci per cell plotted against total time of UVC irradiation (25 J/m^2) (n>50). Bars=SD.*

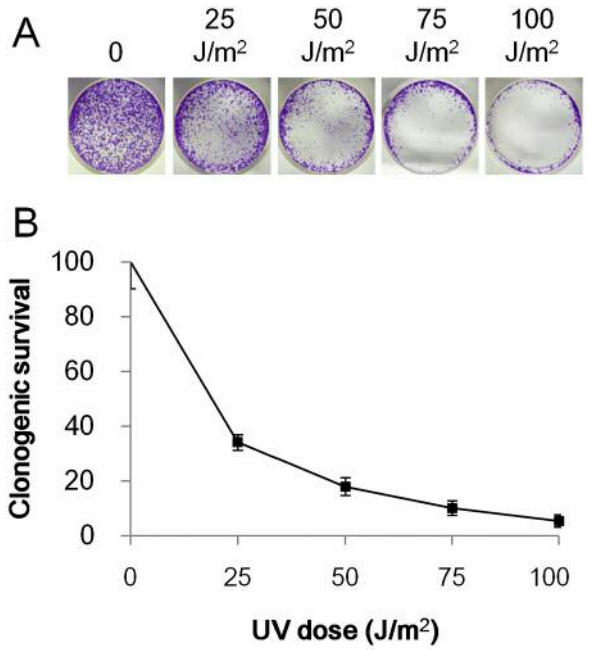

Figure 3. *UVC irradiation induces MCF7-53BP1-GFP cell death. A: Cells were irradiated with UVC (25-100 J/m^2), fixed at 10 days, and stained with crystal violet. B: Clonogenic survival of MCF7-53BP1-GFP cells treated with increasing doses of UVC. Data are the mean±SD (n=6). Clonogenic efficiency of untreated MCF7-53BP1-GFP cells with the control set at 100%.*

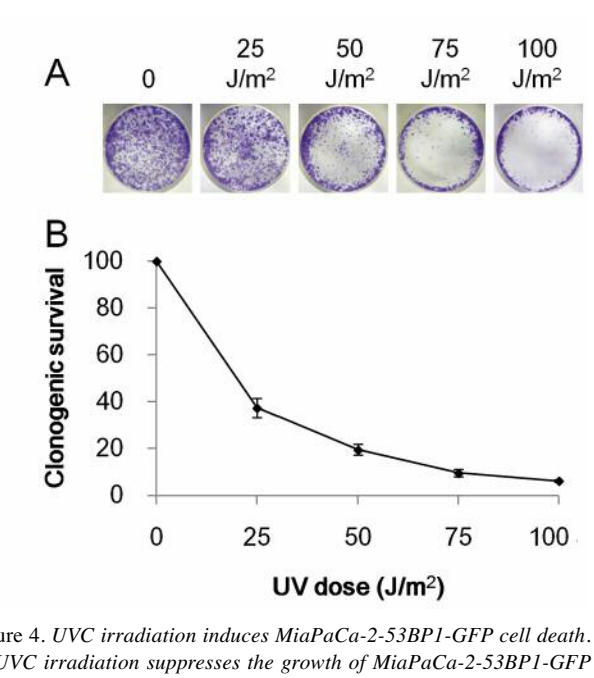

Figure 4. *UVC irradiation induces MiaPaCa-2-53BP1-GFP cell death. A: UVC irradiation suppresses the growth of MiaPaCa-2-53BP1-GFP cells. Cells were irradiated with UVC (25-100 J/m^2), fixed at 10 days, and stained with crystal violet. B: Clonogenic survival of MiaPaCa-2-53BP1-GFP cells treated with increasing doses of UVC. Data are the mean ± SD (n=6). Clonogenic efficiency of untreated MiaPaCa-2-53BP1-GFP cells with the control set at 100%.*

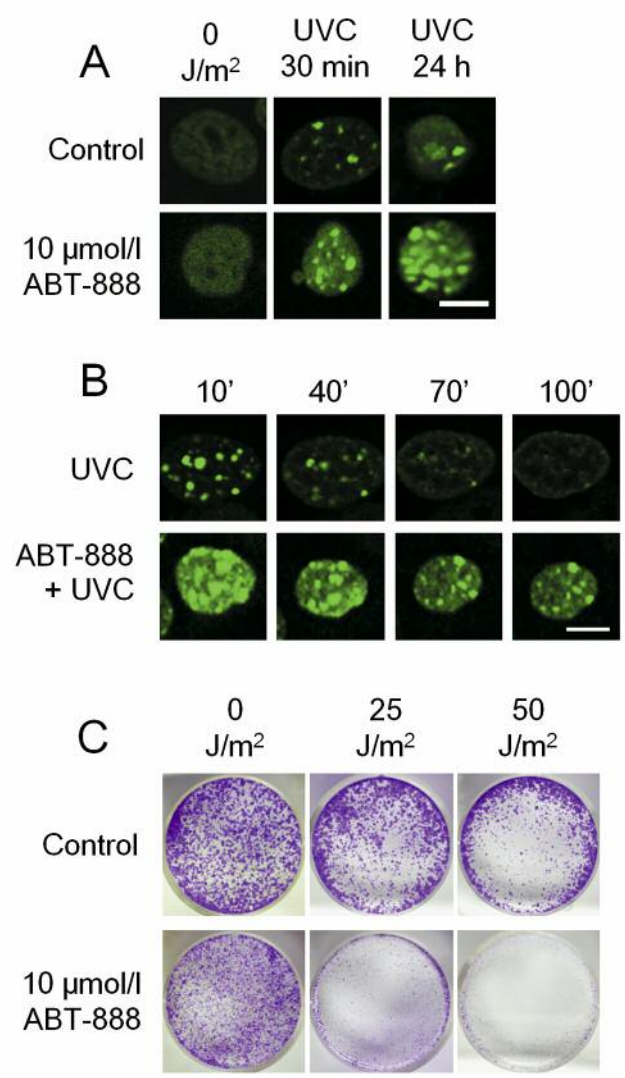

Figure 5. *Poly(ADP-ribose) polymerase 1 (PARP1) inhibitor ABT-888 causes persistence of 53BP1-GFP foci and suppresses cell proliferation of MCF7-53BP1-GFP cells. A: ABT-888 increased the number of residual UVC-induced foci in MCF7-53BP1-GFP cells 24 h after UVC irradiation (25 J/m^2). Cells were pretreated with 10 µmol/l ABT-888 before UVC irradiation. Bar=5 µm. B: Time-course, live-cell imaging of 53BP1-GFP focus formation in MCF7-53BP1-GFP cells treated with UVC with/without ABT-888. Bar=10 µm. C: ABT-888 suppressed the growth of UVC-irradiated MCF7-53BP1-GFP cells. Cells were treated as shown, fixed at 10 days, and stained with crystal violet.*

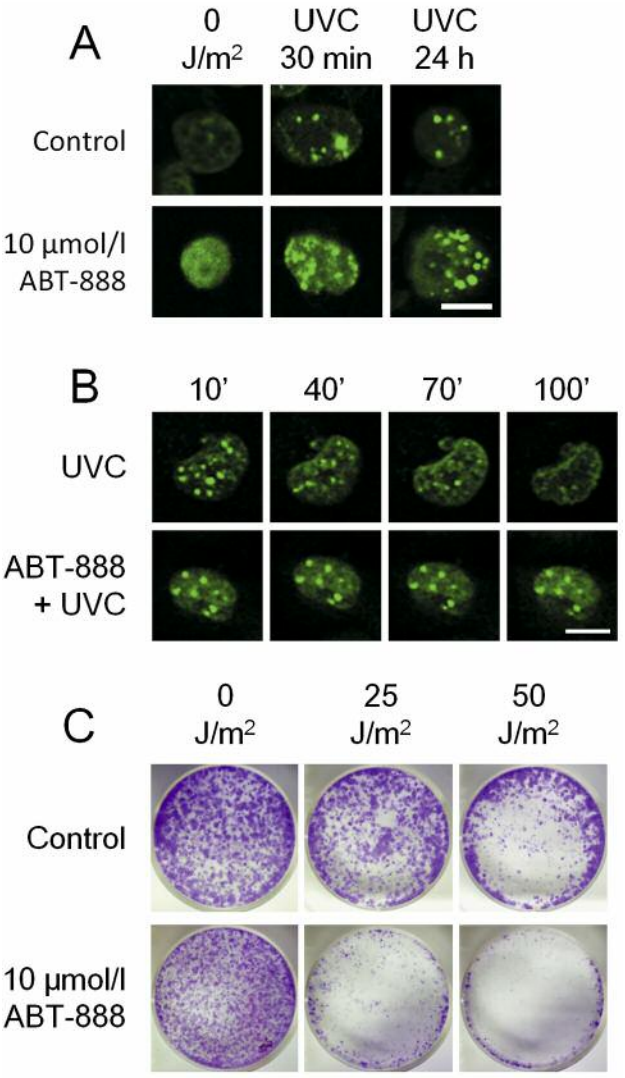

Figure 6. *Poly(ADP-ribose) polymerase 1 (PARP1) inhibitor ABT-888 causes persistence of 53BP1-GFP foci and suppresses cell proliferation of MiaPaCa-2-53BP1-GFP cells. A: ABT-888 increased the number of residual UVC-induced foci in MiaPaCa-2-53BP1-GFP cells 24 h after UVC irradiation (25 J/m^2). Cells were pretreated with 10 µmol/l ABT-888 before UVC irradiation. Bar=5 µm. B: Time-course, live-cell imaging of 53BP1-GFP localization in MiaPaCa-2-53BP1-GFP cells treated with UVC with/without ABT-888. Bar=10 µm. C: ABT-888 suppressed the growth of UVC-irradiated MiaPaCa-2-53BP1-GFP cells. Cells were treated as shown, fixed at 10 days, and stained with crystal violet.*

Enhancement of ionizing-radiation (IR) effects by PARP inhibition has been reported (24-26). IR-induced 53BP1 foci in MCF7 cells persisted with the combination of IR and a PARP inhibitor. Moreover, PARP inhibition increased breast cancer cell senescence both *in vitro* and *in vivo* (24).

PARP inhibitors may be effective as cancer treatment in combination with UV irradiation alone. It has also been shown that expression of a fluorescent protein by cancer cells can enhance killing by UVC (33). This could have curative potential when cancer cells are made fluorescent *in vivo* such as with a GFP-containing telomerase-dependent adenovirus or other fluorophore used for tumor illumination to effect fluorescence-guided surgery (34-39).

Conflicts of Interest

No potential conflicts of interest were disclosed.

Dedication

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

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