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Imaging UVC-Induced DNA Damage Response in Models of Minimal Cancer

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ABSTRACT

We have previously demonstrated that the ultraviolet (UV) light is effective against a variety of cancer cells in vivo as well as in vitro. In the present report, we imaged the DNA damage repair response of minimal cancer after UVC irradiation. DNA-damage repair response to UV irradiation was imaged on tumors growing in 3D culture and in superficial tumors grown in vivo. UV-induced DNA damage repair was imaged with GFP fused to the DNA damage response (DDR)-related chromatin-binding protein 53BP1 in MiaPaCa-2 human pancreatic cancer cells. Three-dimensional Gelfoam® histocultures and confocal imaging enabled 53BP1-GFP nuclear foci to be observed within 1 h after UVC irradiation, indicating the onset of DNA damage repair response. A clonogenic assay showed that UVC inhibited MiaPaCa-2 cell proliferation in a dose-dependent manner, while UVA and UVB showed little effect on cell proliferation. Induction of UV-induced 53BP1-GFP focus formation was limited up to a depth of 40 μm in 3D-culture of MiaPaCa-2 cells. The MiaPaCa-2 cells irradiated by UVC light in a skin-flap mouse model had a significant decrease of tumor growth compared to untreated controls. Our results also demonstrate that 53BP1-GFP is an imageable marker of UV-induced DNA damage repair response of minimal cancer and that UVC is a useful tool for the treatment of residual cancer since UVC can kill superficial cancer cells without damage to deep tissue. J. Cell. Biochem. 114: 2493–2499, 2013.

KEY WORDS: GFP; 53BP1; UVC; DNA DAMAGE; REPAIR RESPONSE; MIAPACA-2; PANCREATIC CANCER; NUDE MICE; SKIN FLAP; THREE-DIMENSIONAL CULTURE IMAGING

Ultraviolet (UV) light irradiation has shown promise in the treatment of cancer in a number of different models. The effect of UVC irradiation was investigated by our laboratory on a model of brain cancer and a model of experimental brain metastasis with cancer cells expressing red fluorescent protein (RFP) in the cytoplasm and green fluorescent protein (GFP) in the nucleus [Momiyama et al., 2013]. UVC irradiation, beamed through the craniotomy open window, induced apoptosis in the cancer cells on the brain as imaged in live mice. UVC irradiation was effective on Lewis lung carcinoma cells (LLC) in the brain and significantly extended survival of the treated mice. In contrast, the U87 glioma was relatively resistant to UVC irradiation.

We also determined the efficacy of UVC irradiation on the growth of murine melanoma expressing GFP in the ear of RFP transgenic nude mice [Yang et al., 2009] expressing RFP in blood vessels using a non-invasive ear-tumor imaging model [Tsai et al., 2010]. UVC irradiation had a direct effect on melanoma growth as well as an anti-angiogenesis effect.

UV-induced cancer cell death was found to be wavelength and dose dependent, as well as cancer cell-line dependent [Kimura et al., 2010]. UVC was most effective. As little as 25 J/m^2 UVC irradiation killed approximately 70% of 143B human osteosarcoma cells expressing GFP and RFP. Cell death began approximately 4 h after irradiation and continued until 10 h after irradiation. UVC exposure also suppressed cancer cell growth in nude mice in a model of minimal residual cancer (MRC) [Kimura et al., 2010].

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After UVC irradiation, the number of fluorescent-protein-expressing cancer cells decreased significantly compared to cells without fluorescent protein [Momiyama et al., 2012].

Previously, Efimova et al. [2010] fused GFP to the chromatin-binding domain of the DNA damage response (DDR)-related checkpoint adapter protein 53BP1 and observed focus formation of this protein after ionizing radiation (IR). Although there are numerous reports about the effect of IR on DDR-related proteins [Efimova et al., 2010], the role of these proteins in the DNA repair response after UV light is poorly understood.

A major problem in surgical oncology is MRC after apparent curative tumor resection. For patients with no evidence of systemic metastases, metastatic relapse often occurs following resection of the primary tumor which is due to cancer cells not removed by the surgeon due to the inability to detect them [Pantel et al., 2009].

In this study, using 53BP1-GFP as a marker of early response to DNA damage, we investigated the efficacy and limitation of UV light as a therapeutic modality for MRC.

MATERIALS AND METHODS

CELL CULTURE AND GENE CONSTRUCTS

GFP fused to the human 53BP1 IRIF-binding domain was cloned into the pLVX-Tight-Puro lentivival vector (Clontech, Mountain View, CA) [Efimova et al., 2010], which was then transduced into the MiaPaCa-2$^{Tet\text{-}On}$ Advanced cell line (Clontech) and cultured in high-glucose DMEM (Invitrogen, Grand Island, NY) with 10% Tet system-approved fetal bovine serum (Clontech). MiaPaCa-2$^{Tet\text{-}On}$ Advanced is certified by Clontech as devived from MiaPaCa-2 (American Type Culture Collection, Manassas, VA) by viral transduction and was used without further authentication. After induction for 48 h with 1 μg/ml doxycycline (Sigma, St. Louis, MO), GFP-positive cells were sorted to establish a stable MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cell line.

CLONOGENIC ASSAY

MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells (1×10^3 cells/dish) were seeded in 35 mm dishes and treated with 1 μg/ml doxycycline for 48 h. Then, the cells were irradiated with various doses of UVA, UVB, and UVC (25–200 J/m^2). After 7 days culture, the colonies were fixed with ethanol and then stained with crystal violet. ImageJ was used to quantify the colonies of the cells.

MICE

Transgenic nude RFP mice (RFP nude mice) (AntiCancer Inc., San Diego, CA) were used in this study [Yang et al., 2009]. Mice were bred and maintained in a barrier facility under HEPA filtration at AntiCancer, Inc. Mice were fed with an autoclaved laboratory rodent diet. All animal studies were conducted in accordance with the principles and procedures outlined in the National Research Council's Guide for the Care and Use of Laboratory Animals under PHS Assurance Number A3873-01.

UV IRRADIATION AND 53BP1-GFP FOCUS FORMATION

For in vitro UV irradiation, the MiaPaCa2$^{Tet\text{-}On}$ 53BP1-GFP cells were cultured in 35 mm dishes and treated with 1 μg/ml doxycycline for 48 h. The cells were irradiated with UV light from the bottom of the chamber using a Benchtop 3UV transilluminator (UVP, LLC, Upland, CA), which emits UVC with an emission peak at 254 nm; UVB with an emission peak at 302 nm; and UVA with an emission peak at 365 nm. For in vivo UV irradiation, a customized UVC pen light (emission peak at 265 nm) (UVP) was used as previously reported [Kimura et al., 2010]. The UV dose was measured with a UVX Radiometer (UVP). 53BP1-GFP focus formation was imaged with the FluoView FV1000 confocal laser microscope (Olympus Corp., Tokyo, Japan) [Uchugonova et al., 2011]. High-resolution images were captured directly on a personal computer (Fujitsu Siemens Computers, Munich, Germany). Images were analyzed with the use of Cell® software (Olympus Biosystems). Focus-positive cells were defined as cells which contained five or more foci.

THREE-DIMENSIONAL GELFOAM® CULTURE

Gelfoam® (Pharmacia & Upjohn Co., Kalamazoo, MI) was cut in $10 \times 10 \times 3$ mm^3 pieces and soaked in DMEM medium with 1 μg/ml doxycycline. MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells (1×10^6) were seeded on hydrated Gelfoam®, in DMEM with 10% FBS and 1 μg/ml doxycycline, with sufficient volume to cover the Gelfoam® in 35 mm dishes. Care was taken so that cells did not float away from the Gelfoam® and scatter in the medium. Forty-eight hours after seeding, the cells were irradiated with 500 J/m^2 UVC. 53BP1-GFP focus formation in the cells at various depths from the surface was imaged with the FV1000 confocal microscope.

PENETRATION OF UVC LIGHT ON EXCISED TUMORS

MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells were injected in RFP nude mice (1×10^6 cells/mouse, s.c.). Once tumors grew to 100 mm^3, 2 mg/ml doxycycline was added to the drinking water for 72 h. After the tumors were excised and sliced, the tumor surface was irradiated with 500 J/m^2 UVC. 53BP1-GFP focus formation in the cells at various depths was imaged with the FV1000.

UVC IRRADIATION SKIN-FLAP CANCER IMAGING MODEL OF MINIMAL RESIDUAL CANCER (MRC)

RFP nude mice [Yang et al., 2009] were anesthetized with a ketamine mixture (10 μl ketamine HCl, 7.6 μl xylazine, 2.4 μl acepromazine maleate, and 10 μl H_2O). To investigate the efficacy of UVC light on a residual cancer model, an arc-shaped incision was made in the abdominal skin, and subcutaneous connective tissue was separated to free the skin flap without injuring the epigastric cranialis artery and vein. MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells (1×10^6 in 10 μl) were sprinkled on the skin flap [Yamauchi et al., 2012]. Irradiation with UVC was carried out 24 h later. The mice were observed 2, 4, and 6 weeks after irradiation. Tumor sizes were compared between the irradiated and non-irradiated groups at 14, 28, and 42 days after irradiation.

STATISTICAL ANALYSIS

The experimental data are expressed as the mean ± SD. Statistical analysis was performed using the Student's *t*-test or one-way analysis of variance (ANOVA) test. *P*-values less than 0.05 were considered statistically significant.

RESULTS

EFFECT OF UVA, UVB, AND UVC ON MIAPACA-2^{TET-ON} 53BP1-GFP CELL PROLIFERATION IN VITRO

To determine if UV-induced cancer cell killing is dose and wavelength dependent, MiaPaCa-2^{Tet-On} 53BP1-GFP cells (1×10^3) were seeded in 35 mm dishes and treated with 1 μg/ml doxycycline for 48 h. The cells were then irradiated with various doses of UVA, UVB, and UVC (25–200 J/m^2). After 7 days culture, the colonies were fixed in ethanol and stained with crystal violet. UVA had no effect on the cells. Low doses of UVB did not affect cell proliferation (Fig. 1). In contrast, 100–200 J/m^2 UVB and 25–200 J/m^2 UVC significantly inhibited cell proliferation ($P < 0.05$). This result indicates that UVC has a strong killing effect on MiaPaCa-2^{Tet-On} 53BP1-GFP cells (Fig. 1).

53BP1-GFP FOCUS FORMATION IN MIAPACA-2^{TET-ON} 53BP1-GFP CELLS IN THREE-DIMENSIONAL GELFOAM® HISTOCULTURE AFTER UVC IRRADIATION

To investigate the depth of penetration by UVC irradiation, 53BP1-GFP focus formation was determined in three-dimensional histoculture using Gelfoam® as a sponge matrix [Leighton, 1951; Freeman and Hoffman, 1986; Vescio et al., 1987; Hoffman, 2010]. One hour after 500 J/m^2 UVC irradiation, 53BP1-GFP focus formation of the cells at each depth was imaged using the FV1000 confocal

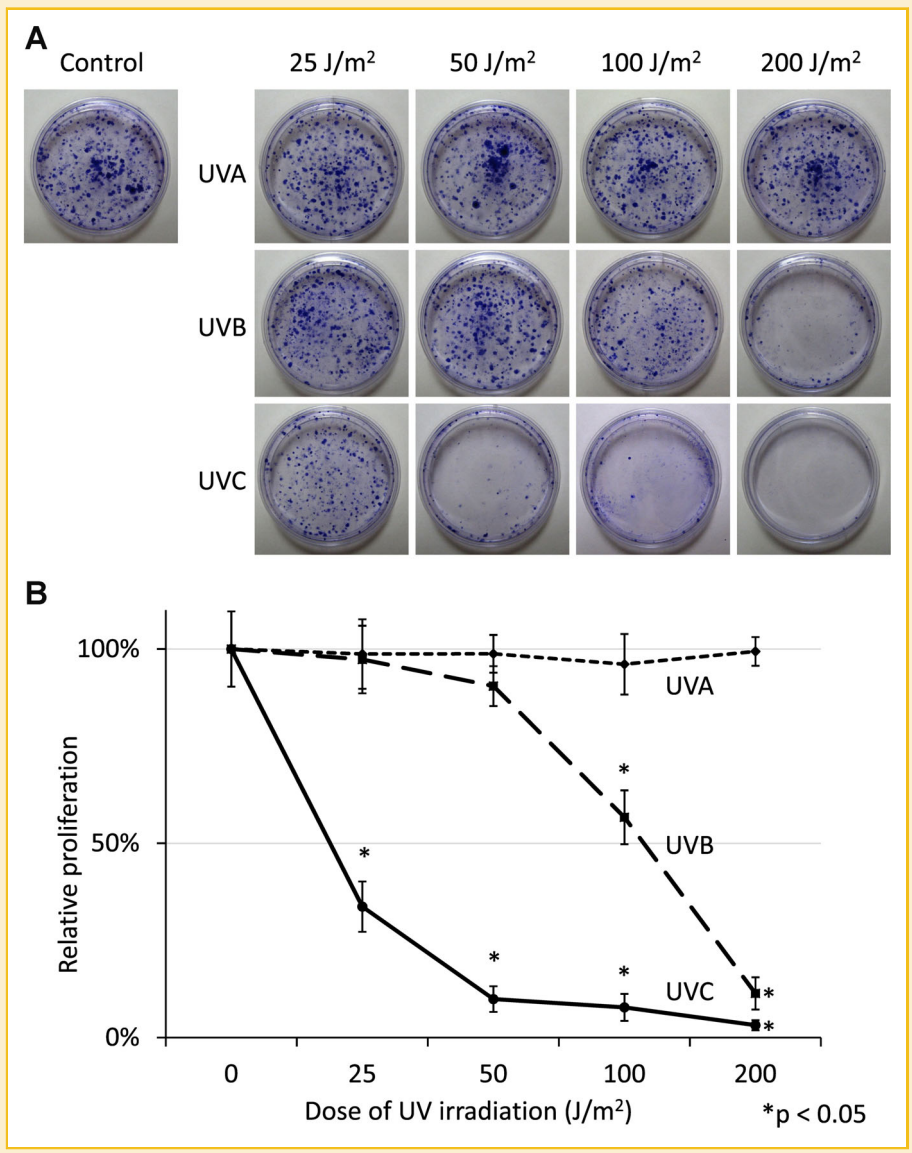

Fig. 1. Effect of UVA, UVB, and UVC on MiaPaCa-2^{Tet-On} 53BP1-GFP cell proliferation in vitro. Seven days after UVA, UVB, and UVC irradiation of MiaPaCa-2^{Tet-On} 53BP1-GFP cells, clonogenic assays were performed. A: Crystal violet-stained dishes of ethanol-fixed MiaPaCa-2^{Tet-On} 53BP1-GFP cells. B: Proliferation-inhibitory effect of UVA, UVB, and UVC. The results of clonogenic assays were analyzed by ImageJ. UVA and low doses of UVB did not affect proliferation of the cells. UVC and high doses of UVB inhibited cell proliferation. The experimental data are expressed is the mean ± SD. Statistical analysis was performed using the ANOVA test. *$P < 0.05$, compared with control. Please see Materials and Methods Section for details.

microscope (Fig. 2). The cells at 20 and 40 μm depth had an increased the extent of focus formation after irradiation ($P < 0.05$). However, there was no significant difference in focus formation in cells at 60 and 80 μm depths and control cells. This result suggests that penetration of UVC is limited to 40 μm depth in Gelfoam®.

53BP1-GFP FOCUS FORMATION IN UV-IRRADIATED EXCISED TUMORS

To confirm the depth of penetration by UVC irradiation in vivo, 53BP1-GFP focus formation was imaged after UVC irradiation of excised tumor tissue. MiaPaCa-2^{Tet-On} 53BP1-GFP cells were injected (s.c.) in RFP nude mice. When the tumor size reached 100 mm^3, the mice were treated with doxycycline for 72 h. The tumor mass was excised, sliced, and the surface irradiated with 500 J/m^2 UVC. One hour after irradiation, 53BP1-GFP focus formation was imaged at various depths from the tumor surface. The cells which were 20 and 40 μm from the tumor surface showed an increased number of 53BP1-GFP foci compared to untreated control, but no increase in focus formation was seen at depths of 60 and 80 μm (Fig. 3). This result indicates that UVC can penetrate up to 40 μm, and this result supports the result observed in three-dimensional Gelfoam® histoculture described above.

EFFICACY OF UVC ON CANCER CELLS SEEDED ON A SKIN FLAP IN NUDE MICE

MiaPaCa-2^{Tet-On} 53BP1-GFP cells were sprinkled on skin flaps in RFP nude mice [Yamauchi et al., 2012]. The mice were divided into control and UVC-treatment groups. Twenty-four hours after cell sprinkling on the skin flaps, the cells were irradiated with 1,950 J/m^2 UVC. In the control group, tumor sizes on days 14, 28, and 42 were 7.4 ± 11.4;

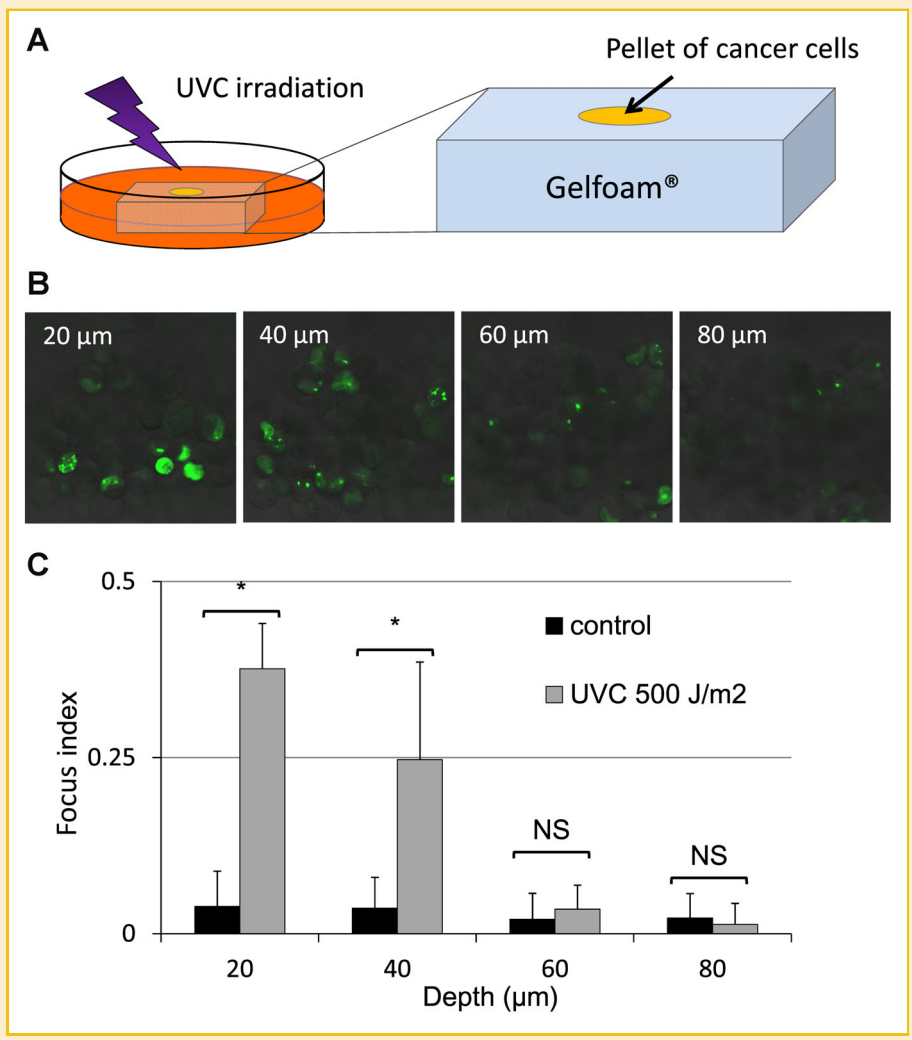

Fig. 2. Penetration of UVC in three-dimensional Gelfoam® histoculture of MiaPaCa-2^{Tet-On} 53BP1-GFP cells. A: MiaPaCa-2^{Tet-On} 53BP1-GFP cells were centrifuged and the cell pellet was put on Gelfoam® and irradiated with UVC. One hour after UVC irradiation, 53BP1-GFP focus formation at the designated depth of Gelfoam® was imaged. B: The UVC-irradiated cells at 20 and 40 μm depth showed increased focus formation compared to untreated controls, but the cells at 60 and 80 μm showed only small numbers of foci. C: There were significant differences in the focus index between the UVC-treated cultures and control cultures at only 20 and 40 μm depth. The experimental data are expressed as the mean ± SD. Statistical analysis was performed using the Student's *t*-test. $^*P < 0.05$, compared with control. Please see Materials and Methods for details.

53.8 ± 46.0; and 281.8 ± 220.1 mm^3, respectively. In the UVC group, tumor sizes on days 14, 28, and 42 were 1.6 ± 2.2; 14.4 ± 17.6; and 70.0 ± 96.8 mm^3, respectively. At days 28 and 42, tumor sizes in the control group were significantly larger than in the UVC-treatment group ($P < 0.05$). No apparent side effects of UVC irradiation were observed (Fig. 4).

DISCUSSION

Although there are several reports on the correlation between IR and 53BP1 focus formation [Efimova et al., 2010], we are only beginning to understand the effects of UV irradiation on 53BP1 focus formation [Miwa et al., 2013].

We previously reported the cell-killing efficacy of UV light on cancer cells expressing fluorecent protiens [Kimura et al., 2010; Tsai et al., 2010; Momiyama et al., 2012,2013]. UV-induced cancer cell death was found to be wave-length and dose dependent, as well as cell-line dependent. We also showed that fluorescent protein expression enhanced UVC cancer cell killing [Momiyama et al., 2012].

For UVC, as little as 25 J/m^2 UVC irradiation killed 70% of dual-color cancer cells expressing GFP in the nucleus and RFP in the cytoplasm [Kimura et al., 2010]. UVC exposure also suppressed cancer cell growth in nude mouse models of MRC [Kimura et al., 2010; Momiyama et al., 2013]. No apparent side effects of UVC exposure were observed. In another previous study, the efficacy of fluorescence-guided UVC irradiation on the growth of murine melanoma expressing GFP in the ear of RFP mice was determined. The GFP-expressing melanoma and RFP-expressing blood vessels from the transgenic mice expressing RFP used as hosts were readily visible using noninvasive imaging. UVC inhibited melanoma growth and also damaged blood vessels in the tumor [Tsai et al., 2010].

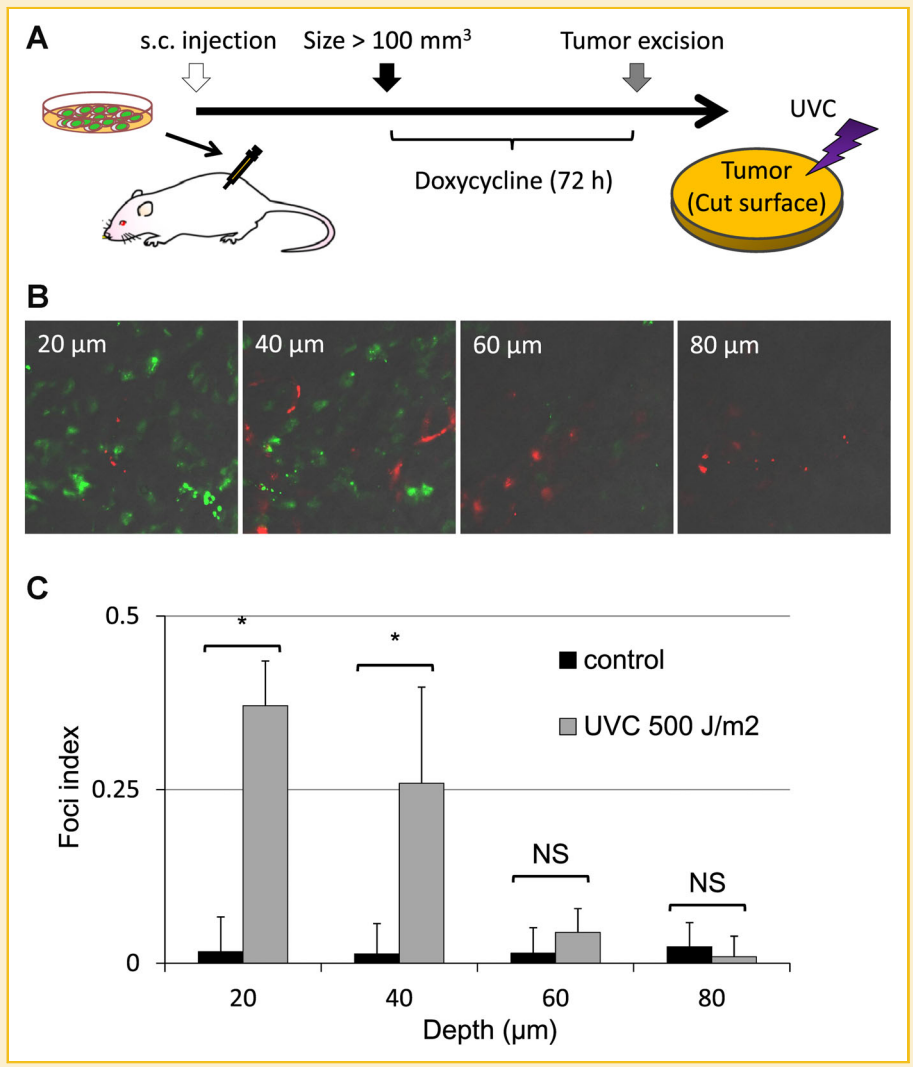

Fig. 3. Penetration of UVC in excised tumors of MiaPaCa-2^{Tet-On} 53BP1-GFP cells. A: MiaPaCa-2^{Tet-On} 53BP1-GFP tumors were excised, sliced, and irradiated with UVC on the tumor surface. B: The UVC-irradiated cells at 20 and 40 μm depth showed increased focus formation, but the UVC-irradiated cells at 60 and 80 μm depth had only a small number of foci. C: There were significant differences in focus formation between the UVC-treated and control tumors only at 20 and 40 μm. The experimental data are expressed as the mean ± SD. Statistical analysis was performed using the Student's *t*-test. $^*P < 0.05$, compared with control. Please see Materials and Methods for details.

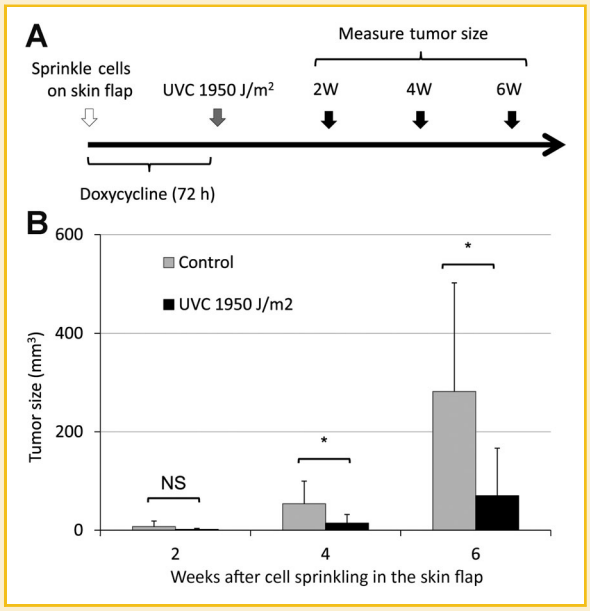

Fig. 4. Efficacy of UVC on cancer MiaPaCa-2^{Tet-On} 53BP1-GFP cells growing on skin flaps. MiaPaCa-2^{Tet-On} 53BP1-GFP cells were sprinkled on skin flaps of RFP nude mice [Yamauchi et al., 2012]. The mice were divided into untreated control and UVC-treated groups. Twenty-four hours after cell sprinkling, the skin flaps were irradiated with 1,950 J/m^2 UVC. In the control group, tumor sizes at days 14, 28, and 42 were 7.4, 53.8, and 281.8 mm^3, respectively. In the UVC group, tumor sizes at days 14, 28, and 42 were 1.6, 14.4, and 70.0 mm^3, respectively. At days 28 and 42 after cell sprinkling, tumor sizes in the control group were significantly larger than in the UVC-treatment group ($P < 0.05$). The experimental data are expressed as the mean ± SD. Statistical analysis was performed using the Student's *t*-test. $^*P < 0.05$, compared with control. Please see Materials and Methods for details.

The results of the present study demonstrate that 53BP1-GFP is an imageable in vivo marker of UV-induced DNA damage repair.

The results of the present study also indicate that UVC is a useful tool for the treatment of residual cancer since UVC can kill superficial cancer cells up to a depth of 40 μm without damage to deep tissue.

OBP-401, a telomerase-dependent, replication-competent adenovirus expressing GFP was used to label tumors in situ [Kishimoto et al., 2009]. GFP has been shown to be a powerful in vivo imaging reporter [Chishima et al., 1997; Yang et al., 2000; Hoffman, 2005, 2012; Hoffman and Yang, 2006a,b,c]. In an intraperitoneal model of disseminated HCT-116 human colon cancer labeled with GFP by OBP-401, fluorescence-guided surgery enabled resection of tumor nodules [Kishimoto et al., 2009; Bouvet and Hoffman, 2011].

However, technical problems remain to remove all cancer cells by fluorescence-guided surgery. Recurrence after fluorescence-guided surgery of tumors labeled with GFP in vivo with OBP-401 could be imaged. Recurrent tumor nodules brightly expressed GFP, indicating that initial OBP-401-GFP labeling of peritoneal disease was genetically stable, such that proliferating residual cancer cells still express GFP [Kishimoto et al., 2011]. An important application of UV irradiation would be to sterilize the bed after fluorescence-guided surgery [Hoffman and Bouvet, 2012].

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