ARTICLE
Journal of Cellular Biochemistry 115:1724–1728 (2014)

Journal of Cellular Biochemistry

Comparison of UVB and UVC Effects on the DNA Damage-Response Protein 53BP1 in Human Pancreatic Cancer

Fuminari Uehara,[1,2,3] Shinji Miwa,[1,2] Yasunori Tome,[1,2,3] Yukihiko Hiroshima,[1,2] Shuya Yano,[1,2] Mako Yamamoto,[1] Elena Efimova,[4] Yasunori Matsumoto,[1] Hiroki Maehara,[3] Michael Bouvet,[2] Fuminori Kanaya,[3] and Robert M. Hoffman[1,2*]

[1]*AntiCancer, Inc., 7917 Ostrow Street, San Diego, California 92111*

[2]*Department of Surgery, University of California, 200 West Arbor Drive, San Diego, California 92103*

[3]*Department of Orthopedic Surgery, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0125, Japan*

[4]*Ludwig Center for Metastasis Research, University of Chicago, Chicago, Illinois*

ABSTRACT

We have previously demonstrated that ultraviolet (UV) light is effective against a variety of cancer cells expressing fluorescent proteins in vivo as well as in vitro. In the present report, we compared the DNA damage repair (DDR) response of pancreatic cancer cells after UVB or UVC irradiation. The UV-induced DNA damage repair was imaged with green fluorescent protein (GFP) fused to the DDR-related chromatin-binding protein 53BP1 in MiaPaCa-2 human pancreatic cancer cells growing in 3D Gelfoam® histoculture and in superficial tumors grown in nude mice. 53BP1-GFP forms foci during DNA damage repair. A clonogenic assay in 2D monolayer culture initially showed that UVC and UVB inhibited MiaPaCa-2 cell proliferation in a dose-dependent manner, with UVC having more efficacy. Three-dimensional Gelfoam® histocultures and confocal imaging enabled 53BP1-GFP foci to be observed within 1 h after UV irradiation, indicating the onset of DDR response. UVB-induced 53BP1-GFP focus formation was observed up to a depth of 120 μm in MiaPaCa-2 cells on Gelfoam® compared to 80 μm for UVC. UVB-induced 53BP1-GFP focus formation was observed up to a depth of 80 μm in MiaPaCa-2 cells, implanted within skin flaps in mice, at a significantly greater extent than UVC. MiaPaCa-2 cells irradiated by UVB or UVC in the skin-flap mouse model had a significant decrease in tumor growth compared to untreated controls with UVB having more efficacy than UVC. Our results demonstrate that UVB has greater tissue penetration than UVC because of its longer wavelength and has clinical potential for eradicating superficial cancer. J. Cell. Biochem. 115: 1724–1728, 2014. © 2014 Wiley Periodicals, Inc.

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

We have previously shown that UVC is more efficacious against cancer cells expressing a fluorescent protein than on cells not expressing a fluorescent protein [Momiyama et al., 2012]. After UVC irradiation, the number of fluorescent-protein-expressing cancer cells decreased significantly compared to cells without fluorescent protein [Momiyama et al., 2012].

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 a craniotomy open window, was effective on GFP-expressing Lewis

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

The authors have a conflict of interest in regard to this study.

Grant sponsor: NCI; Grant numbers: CA132971, CA164492.

*Correspondence to: Robert M. Hoffman, Ph.D., AntiCancer. Inc., 7917 Ostrow Street, San Diego, CA 92111.
E-mail: all@anticancer.com

Manuscript Received: 23 April 2014; Manuscript Accepted: 8 May 2014

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 12 May 2014

DOI 10.1002/jcb.24837 • © 2014 Wiley Periodicals, Inc.

lung carcinoma cells (LLC) in the brain but not U87 glioma. We also determined the efficacy of UVC irradiation on the growth of murine melanoma expressing GFP in the ear of RFP transgenic nude mice expressing RFP in blood vessels using a non-invasive ear-tumor imaging model [Tsai et al., 2010]. UVC irradiation inhibited melanoma growth as well as having an anti-angiogenesis effect.

As little as 25 J/m^2 UVC irradiation killed approximately 70% of 143B human osteosarcoma cells expressing GFP and RFP [Kimura et al., 2010]. UVC exposure also suppressed cancer cell growth in nude mice in a model of minimal residual cancer (MRC) [Kimura et al., 2010].

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).

We imaged the DNA damage repair response of minimal cancer after UVC irradiation. UV-induced DNA damage repair was imaged by focus formation of 53BP1-GFP in MiaPaCa-2 human pancreatic cancer cells in three-dimensional Gelfoam® histocultures with confocal microscopy [Miwa et al., 2013a,b].

In the present study, we compared UVB and UVC using 53BP1-GFP focus formation as a marker of early response to DNA damage in Gelfoam® histoculture and superficial tumors of MiaPaCa-2 human pancreatic cancer cells.

MATERIALS AND METHODS

CELL CULTURE AND GENE CONSTRUCTS

GFP fused to the human 53BP1 IR-induced foci (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 [Miwa et al., 2013a,b].

CLONOGENIC ASSAY

MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells (1×10^3 cells/dish) were seeded in 35 mm dishes and treated with doxycycline (1 μg/ml) for 48 h. Then, the cells were irradiated with various doses of UVA, UVB, and UVC (100–500 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 [Miwa et al., 2013a,b].

MICE

Transgenic nude RFP mice (RFP nude mice) (AntiCancer, Inc., San Diego, CA) were used in this study [Yang et al., 2009]. Mice were fed 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&tapos;s Guide for the Care and Use of Laboratory Animals under PHS Assurance Number A3873-01.

UV IRRADIATION AND 53BP1-GFP FOCUS FORMATION

MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells were cultured in 35 mm dishes and treated with doxycycline (1 μg/ml) 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. The UV dose was measured with a UVX Radiometer (UVP). 53BP1-GFP focus formation was imaged with a FluoView FV1000 confocal laser microscope (Olympus Corp., Tokyo, Japan) [Miwa et al., 2013a,b]. 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 $20 \times 20 \times 3$ mm^3 pieces and soaked in DMEM medium with doxycycline (1 μg/ml). MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells (1×10^6) were seeded on hydrated Gelfoam®, in DMEM with 10% FBS and doxycycline (1 μg/ml), with sufficient volume to cover the Gelfoam® in 35 mm dishes [Hoffman, 2010, 2013]. Forty-eight hours after seeding, the cells were irradiated with UVB or UVC (500 J/m^2). 53BP1-GFP focus formation was imaged in the cells at various depths from the surface with the FV1000 confocal microscope [Miwa et al., 2013a].

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, doxycycline (2 μg/ml) was added to the drinking water for 72 h. After the tumors were excised and sliced, the tumor surface was irradiated with UVB or UVC (500 J/m^2). 53BP1-GFP focus formation in the cells at various depths was imaged with the FV1000. Focus indexes were compared between UVB- and UVC-treated and non-irradiated tumors [Miwa et al., 2013a].

SKIN FLAP IMAGING MODEL OF MINIMAL RESIDUAL CANCER (MRC)

RFP nude mice were first anesthetized with the ketamine mixture (10 μl ketamine HCl, 7.6 μl xylazine, 2.4 μl acepromazine maleate, and 20 μl H_2O). To compare the efficacy of UVB and UVC 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 vessels. MiaPaCa-2$^{Tet\text{-}On}$ 53BP1-GFP cells (1×10^6 in 10 μl) were sprinkled on the skin flap [Yamauchi et al., 2012; Miwa et al., 2013a]. Irradiation with UVB and UVC was carried out 24 h later. The mice were observed 2, 4, 6, and 8 weeks after irradiation. Tumor sizes were compared between the irradiated by UVB and UVC and non-irradiated groups at 2, 4, 6, and 8 weeks after irradiation.

STATISTICAL ANALYSIS

The experimental data are expressed as the mean ± SD. Statistical analysis was performed using the one-way analysis of variance (ANOVA) test. *P* values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

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

To investigate whether 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 doxycycline for 48 h. The cells were then irradiated with various doses of UVA, UVB, and UVC (100–500 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 (Fig. 1). In contrast, UVB and UVC irradiation (100–500 J/m^2) significantly inhibited cell proliferation ($P < 0.05$). This result indicates that UVB and UVC have 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 UVB AND UVC IRRADIATION

To investigate the depth of penetration by UVB and 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, 2013; Miwa et al., 2013a; Tome et al., 2014]. One hour after UVB or UVC irradiation (500 J/m^2), 53BP1-GFP focus formation of the cells at various depths was imaged using a FV1000 confocal microscope (Fig. 2). MiaPaCa-2^{Tet-On} 53BP1-GFP cells at 0 and 40 μm depth had increased focus formation after UVC irradiation ($P < 0.05$). However, there was no significant difference in focus formation in cells at 80 and 120 μm depths between UVC-irradiated and control cells. In contrast, UVB-treated cultures had 53BPl-GFP foci at depths of 120 μm in Gelfoam®. There are significant differences in the focus index between UVB-treated cultures and control cultures at 0, 40, 80, and 120 μm depths. Moreover, UVB-treated cultures had a significantly increased focus index compared to UVC-treated cultures at 120 μm depth. This result demonstrated that penetration

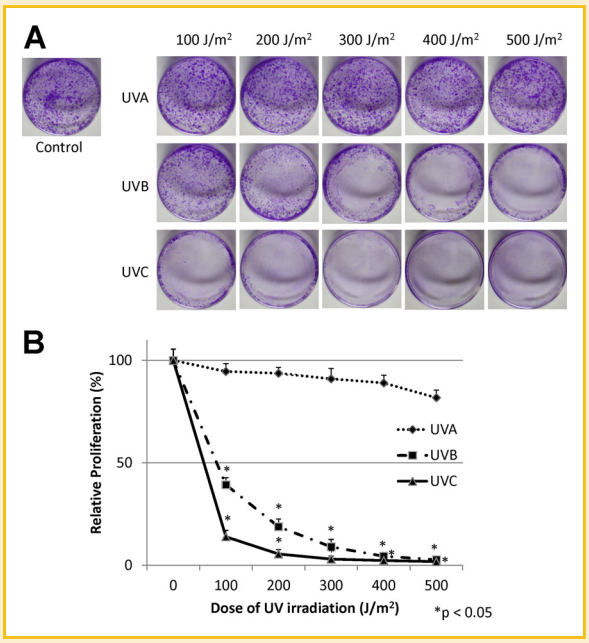

Fig. 1. Effect of UVA, UVB, and UVC on MiaPaca-2^{Tet-On} 53BP1 cell proliferation in vitro. Seven days after UVA, UVB, and UVC irradiation of MiaPaca-2^{Tet-On} 53BP1 cells, clonogenic assays were performed. A: Crystal violet-stained dishes of ethanol-fixed MiaPaca-2^{Tet-On} 53BP1 cells. B: Proliferation-inhibitory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

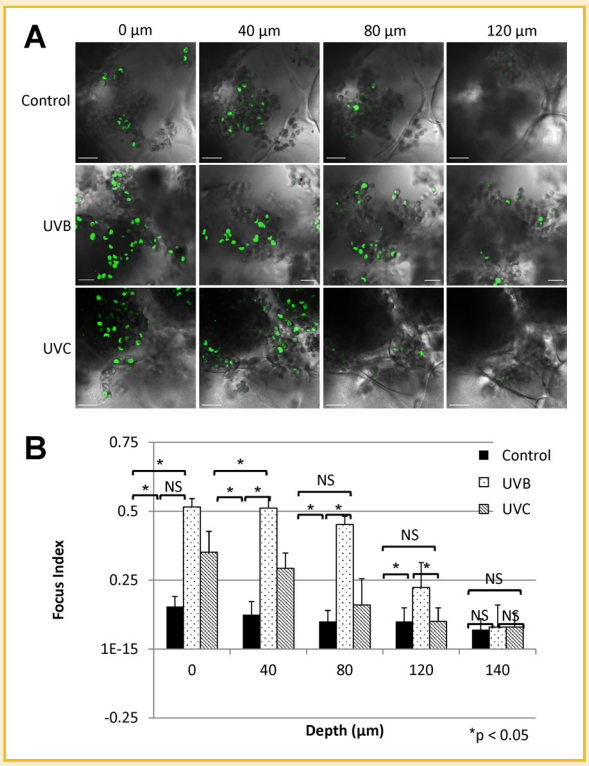

Fig. 2. Penetration of UVB and UVC in three-dimentional Gelfoam® histoculture of MiaPaca-2^{Tet-On} 53BP1-GFP cells. MiaPaca-2^{Tet-On} 53BP1 cells were centrifuged and the cell pellet was placed on Gelfoam® and irradiated with UVB and UVC. A: One hour after UV irradiation, 53BP1-GFP focus formation was compared to untreated controls. UVB-treated cultures had foci at 120 μm depth in Gelfoam®. In contrast, UVC and untreated cultures at 80 and 120 μm had only small numbers of foci. B: There are significant differences in the focus index between the UVC-treated cultures and control cultures only at 0 and 40 μm depth. In contrast, there were significant differences in the focus index between UVB-treated cultures and control cultures at 0, 40, 80, and 120 μm depths. Moreover, UVB-treated cultures had a significantly increased focus index compared to UVC-treated cultures at 120 μm depth. The experimental data are expressed as the mean ± SD. Statistical analysis was performed using the ANOVA test. *$P < 0.05$. Bars = 50 μm.

of UVC is limited to 40 mm depth in Gelfoam®. UVB can penetrate to at least 120 μm depth in Gelfoam®.

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

53BP1-GFP focus formation was imaged after UVB or 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 UVB or UVC (500 J/m^2). One hour after irradiation, 53BP1-GFP focus formation was imaged at various depths from the tumor surface. The UVC-irradiated cells at 0 and 40 μm depth showed increased focus formation, but the UVC-irradiated cells and untreated cells at 80 and 120 μm depth had only a small number of foci. In contrast, UVB-irradiated cells at 80 μm depth showed increased focus formation. Only a small number of foci were detected at 120 μm depth. There were significant differences in the focus index between the UVC-treated tumors and control tumors at 0 and 40 μm depth. In contrast, there were significant differences in the focus index between UVB-treated tumors and control tumors at 0, 40, 80, and 120 μm depths. Moreover, UVB-treated cultures had a significantly increased focus index compared to UVC-treated tumors at 80 μm depth. This result indicates that UVC can penetrate up to 40 μm and UVB can penetrate at least to 80 μm in excised tumors (Fig. 3).

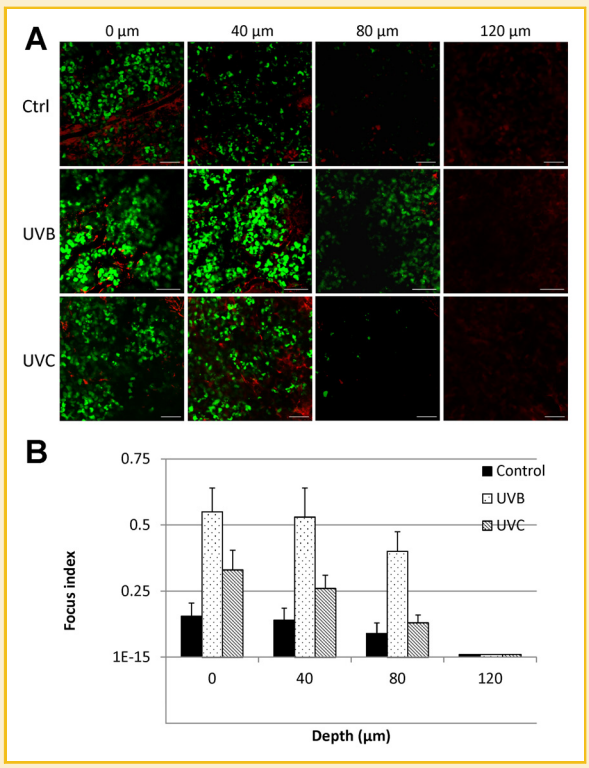

Fig. 3. Penetration of UVB and UVC of MiaPaca-2^{Tet-On} 53BP1-GFP in excised tumors. MiaPaca-2^{Tet-On} 53BP1-GFP tumors were excised, sliced, and irradiated with UVB and UVC on the tumor surface. A: UVC-irradiated cells at 0 and 40 μm depth showed increased focus formation. However, the UVC-irradiated cells and untreated cells at 80 and 120 μm depth had only a small number of foci. In contrast, UVB-irradiated cells at 80 μm depth showed increased focus formation compared to untreated controls for all statistically significant data. B: There were significant differences in the focus index between the UVC-treated cultures and control cultures at only 0 and 40 μm depth. In contrast, there were significant differences in the focus index between UVB-treated cultures and control cultures at 0, 40, and 80 μm depth. Moreover, UVB-treated cultures had significantly increased focus index compared to UVC-treated cultures at 80 μm depth. The experimental data are expressed as the mean ± SD. Statistical analysis was performed using the ANOVA test. $P < 0.05$. Bars = 50 μm.

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

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, UVB-treated, and UVC-treated groups. Twenty-four hours after cell sprinkling, the skin flaps were irradiated with UVB or UVC (1,950 J/m^2). In the control group, tumor sizes at days 14, 28, 42, and 56 were 56.5 ± 45.4, 142.9 ± 116.1, 363.4 ± 249.5,

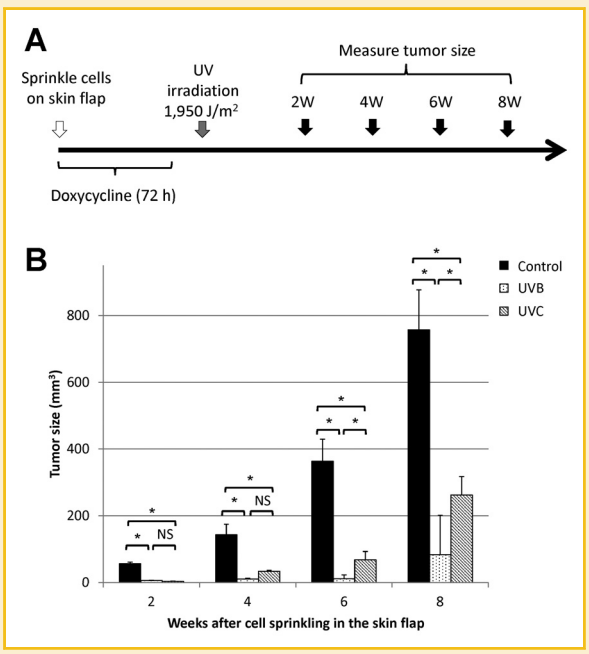

Fig. 4. Efficacy of UVB and UVC on MiaPaca-2^{Tet-On} 53BP1-GFP cells growing on skin flaps. A: 53BP1-GFP cells were sprinkled on skin flaps of RFP nude mice [Yamauchi et al., 2012]. The mice were divided into untreated control, UVB-treated, and UVC-treated groups. Twenty-four hours after cell sprinkling, the skin flaps were irradiated with UVB or UVC (1,950 J/m^2). B: In the control group, tumor sizes at days 14, 28, 42, and 56 were 56.5, 142.9, 363.4, and 757.4 mm^3, respectively. In the UVB group, tumor sizes at days 14, 28, 42, and 56 were 6.1, 10.7, 11.5, and 83.7 mm^3, respectively. In the UVB group, tumor sizes at days 14, 28, 42, and 56 were 3.7, 33.8, 68.1, and 262.5 mm^3, respectively. At days 14, 28, 42, and 56 after cell sprinkling, tumor sizes in the control group were significantly larger than UVB- and UVC-treated groups [$^*P < 0.05$]. Moreover, at days 42 and 56 after cell sprinkling, tumor sizes in the UVC-treated group were significantly larger than UVB-treated group [$^*P < 0.05$]. The experimental data are expressed as the mean ± SD. Statistical analysis was performed using the ANOVA test. Bars = 50 μm.

and $757.4 \pm 470.6\ mm^3$, respectively. In the UVB group, tumor sizes at days 14, 28, 42, and 56 were 6.1 ± 6.5, 10.7 ± 5.5, 11.5 ± 19.2, and $83.7 \pm 117.1\ mm^3$, respectively. In the UVC group, tumor sizes at days 14, 28, 42, and 56 were 3.7 ± 6.9, 33.8 ± 25.1, 68.1 ± 55.0, and $262.5 \pm 204.0\ mm^3$, respectively. At days 14, 28, 42, and 56 after cell sprinkling, tumor sizes in the control group were significantly larger than UVB-treated and UVC-treated groups [$P < 0.05$]. Moreover, at days 42 and 56 after cell sprinkling, tumor sizes in the UVC-treated group were significantly larger than the UVB-treated group [$P < 0.05$]. This result indicated that UVB irradiation had more anti-tumor efficacy than UVC irradiation. No apparent side effects of UV irradiation were observed. UVB can kill superficial cancer cells up to a depth of 80 mm without damage to deep tissue (Fig. 4).

Our results demonstrated that both UVB and UVC are useful tools for the treatment of residual cancer, and UVB was more effective than UVC due to greater tissue penetration of UVB due to its longer wavelength.

In a recent study [Bald et al., 2014], it was found that repetitive UV exposure of primary cutaneous melanomas in a genetically engineered mouse model promotes metastatic progression. UV irradiation enhanced the expansion of cancer cells along abluminal blood vessel surfaces, a process called angiotropism, a process observed by us earlier [Yamauchi et al., 2006], and also increased the number of lung metastases. Thus, UV may promote as well as kill cancer cells including melanoma [Tsai et al., 2010].

ACKNOWLEDGMENTS

This work was supported in part by NCI grants CA126023 and CA164492.

REFERENCES

Bald T, Quast T, Landsberg J, Rogava M, Glodde N, Lopez-Ramos D, Kohlmeyer J, Riesenberg S, van den Boorn-Konijnenberg D, Hömig-Hölzel C, Reuten R, Schadow B, Weighardt H, Wenzel D, Helfrich I, Schadendorf D, Bloch W, Bianchi ME, Lugassy C, Barnhill RL, Koch M, Fleischmann BK, Förster I, Kastenmüller W, Kolanus W, Hölzel M, Gaffal E, Tüting T. 2014. Ultraviolet-radiation-induced inflammation promotes angiotropism and metastasis in melanoma. Nature 507:109–113.

Efimova EV, Mauceri HJ, Golden DW, Labay E, Bindokas VP, Darga TE, Chakraborty C, Barreto-Andrade JC, Crawley C, Sutton HG, Kron SJ, Weichselbaum RR. 2010. Poly (ADP-ribose) polymerase inhibitor induces accelerated senescence in irradiated breast cancer cells and tumors. Cancer Res 70:6277–6282.

Freeman AE, Hoffman RM. 1986. In vivo-like growth of human tumors in vitro. Proc Natl Acad Sci USA 83:2694–2698.

Hoffman RM. 2010. Histocultures and their use. In: Encyclopedia of life sciences. Chichester: John Wiley & Sons Ltd. Published online: Doi: 10.1002/9780470015902.a0002573.pub2.

Hoffman RM. 2013. Tissue culture. In: Brenner S, Miller JH, editors. Brenner's encyclopedia of genetics, 2nd Ed., Vol. 7. London, UK: Elsevier. pp 73–76.

Kimura H, Lee C, Hayashi K, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Bouvet M, Hoffman RM. 2010. UV light killing efficacy of fluorescent protein-expressing cancer cells in vitro and in vivo. J Cell Biochem 110:1439–1446.

Leighton J. 1951. A sponge matrix method for tissue culture; formation of organized aggregates of cells in vitro. J Natl Cancer Inst 12:545–561.

Miwa S, Tome Y, Yano S, Hiroshima Y, Uehara F, Mii S, Kimura H, Hayashi K, Tsuchiya H, Bouvet M, Efimova EV, Hoffman RM. 2013. Single cell time-lapse imaging of focus formation by the DNA damage-response protein 53BP1 after UVC irradiation of human pancreatic cancer cells. Anticancer Res 33:1373–1378.

Miwa S, Yano S, Hiroshima Y, Tome Y, Uehara F, Mii S, Efimova EV, Kimura H, Hayashi K, Tsuchiya H, Hoffman RM. 2013. Imaging UVC-induced DNA damage response in models of minimal cancer. J Cell Biochem 114:2493–2499.

Momiyama M, Suetsugu A, Kimura H, Kishimoto H, Aki R, Yamada A, Sakurada H, Chishima T, Bouvet M, Bulgakova NN, Endo I, Hoffman RM. 2012. Fluorescent proteins enhance UVC PDT of cancer cells. Anticancer Res 32:4327–4330.

Momiyama M, Suetsugu A, Kimura H, Kishimoto H, Aki R, Yamada A, Sakurada H, Chishima T, Bouvet M, Endo I, Hoffman RM. 2013. Imaging the efficacy of UVC irradiation on superficial brain tumors and metastasis in live mice at the subcellular level. J Cell Biochem 114:428–434.

Tsai MH, Aki R, Amoh Y, Hoffman RM, Katsuoka K, Kimura H, Lee C, Chang CH. 2010. GFP-fluorescence-guided UVC irradiation inhibits melanoma growth and angiogenesis in nude mice. Anticancer Res 30:3291–3294.

Tome Y, Uehara F, Mii S, Yano S, Zhang L, Sugimoto N, Maehara H, Bouvet M, Tsuchiya H, Kanaya F, Hoffman RM. 2014. 3-Dimensional tissue is formed from cancer cells in vitro on Gelfoam®, but not on Matrigel™. J Cell Biochem [Epub ahead of print]. DOI: 10.1002/jcb.24780.

Vescio RA, Redfern CH, Nelson TJ, Ugoretz S, Stern PH, Hoffman RM. 1987. In vivo-like drug responses of human tumors growing in three-dimensional gel-supported, primary culture. Proc Natl Acad Sci USA 84:5029–5033.

Yamauchi K, Yang M, Jiang P, Xu M, Yamamoto N, Tsuchiya H, Tomita K, Moossa AR, Bouvet M, Hoffman RM. 2006. Development of real-time subcellular dynamic multicolor imaging of cancer-cell trafficking in live mice with a variable-magnification whole-mouse imaging system. Cancer Res 66:4208–4214.

Yamauchi K, Tome Y, Yamamoto N, Hayashi K, Kimura H, Tsuchiya H, Tomita K, Bouvet M, Hoffman RM. 2012. Color-coded real-time subcellular fluorescence imaging of the interaction between cancer and host cells in live mice. Anticancer Res 32:39–43.

Yang M, Reynoso J, Bouvet M, Hoffman RM. 2009. A transgenic red fluorescent protein-expressing nude mouse for color-coded imaging of the tumor microenvironment. J Cell Biochem 106:279–284.