Single Cell Time-lapse Imaging of Focus Formation by the DNA Damage-Response Protein 53BP1 after UVC Irradiation of Human Pancreatic Cancer Cells

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Abstract. *We have previously demonstrated that ultraviolet (UV) light treatment is effective against various types of cancer cells expressing fluorescent proteins. In order to further understand the efficacy of UV treatment of cancer cells, we determined the kinetics of focus formation by imaging of a DNA damage-response (DDR) protein after UVC irradiation of human pancreatic cancer cells. A fusion protein consisting of the DDR protein 53BP1 and green fluorescent protein (GFP) (GFP-53BP1) was used as a live-cell imaging marker for cellular response after UVC irradiation. GFP-53BP1 foci were observed after UVC irradiation of MiaPaCa-2 human pancreatic cancer cells. During live-cell imaging, GFP-53BP1 foci were observed in the cells within 15 min after UVC irradiation, and some of the foci remained stable for at least three hours. GFP-53BP1 focus formation was observed in the pancreatic-cancer cells irradiated by 25-200 J/m^2 UVC. Our results indicate that an early response to DNA damage caused by UVC irradiation can be visualized by increased GFP-53BP1 focus formation by pancreatic cancer cells.*

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Efimova *et al*. (1) fused green fluorescent protein (GFP) to the chromatin-binding domain of the checkpoint adapter and DNA damage-response (DDR) protein 53BP1 as a live-cell imaging reporter for DNA repair in breast cancer cells after ionizing radiation (IR). Although there are numerous reports about the effect of ionizing irradiation on DDR proteins such as 53BP1 (1), the role of these proteins in repair after irradiation with ultraviolet (UV) light is poorly understood.

We previously investigated the cell-killing efficacy of UV light on cancer cells expressing GFP in the nucleus and red fluorescent protein (RFP) in the cytoplasm (dual-color cells) (2). After exposure to various doses of UVA, UVB, or UVC, apoptotic and viable cells were quantitated under fluorescence microscopy using dual-color 143B human osteosarcoma cells, HT-1080 human fibrosarcoma cells, mouse Lewis lung carcinoma (LLC), and XPA-1 human pancreatic cancer cells *in vitro*. UV-induced cancer cell death was found to be wavelength and dose-dependent, as well as cell-line dependent. UVC was most effective. As little as 25 J/m^2 UVC irradiation killed approximately 70% of the 143B dual-color cells. UVC exposure also suppressed cancer cell growth in nude mice in a model of minimal residual cancer using LLC cells.

We also determined the efficacy of UVC irradiation on the growth of murine melanoma expressing GFP in the ear of RFP transgenic nude mice using a non-invasive ear-tumor imaging model (3). The GFP-expressing melanoma and RFP-expressing blood vessels from the transgenic mice expressing RFP used as hosts were readily visible using non-invasive color-coded imaging. UVC irradiation had a direct effect on melanoma growth as well as an anti-angiogenesis effect.

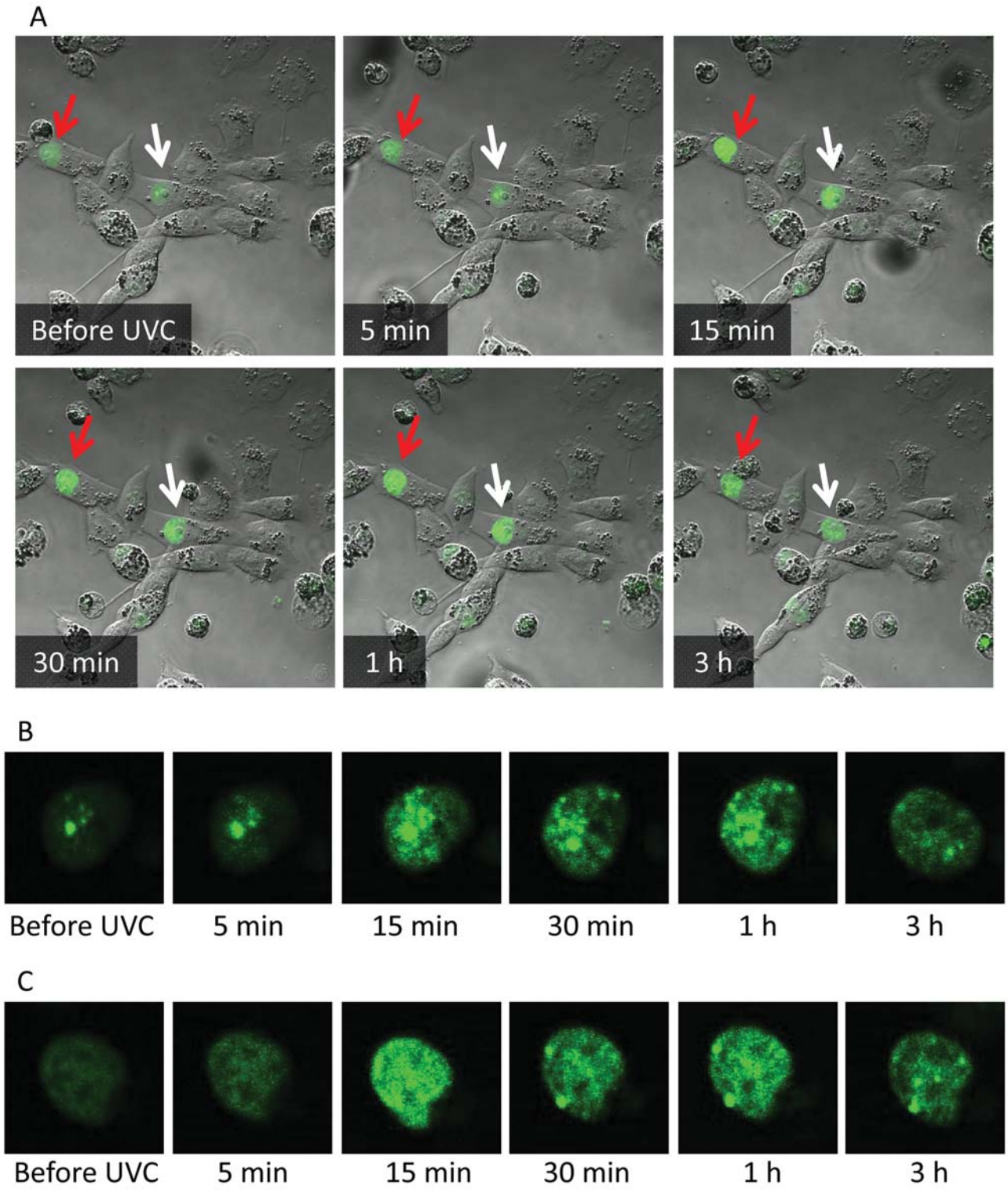

Figure 1. *Time-lapse imaging of GFP-53BP1 focus formation after ultraviolet light (UVC) irradiation of MiaPaCa2$^{Tet\text{-}On}$ GFP-53BP1 cells. MiaPaCa2$^{Tet\text{-}On}$ GFP-53BP1 cells were seeded in a 35 mm dish and treated with 1 μg/ml doxycycline for 48 h. The cells were irradiated with 300 J/m^2 UVC. GFP-53BP1 focus formation was observed with an FV1000 confocal microscope (Olympus). Cells exhibited focus formation within 15 min after irradiation (arrows). A: Bright-field and fluorescence low-magnification (×20) images of the time course of focus formation after UVC irradiation. B: High magnification (×40) of the time course of focus formation, after UVC irradiation, in the cell (white arrows) in A. C: High-magnification images of the time course of focus formation, after UVC irradiation, in the cell in A (red arrows).*

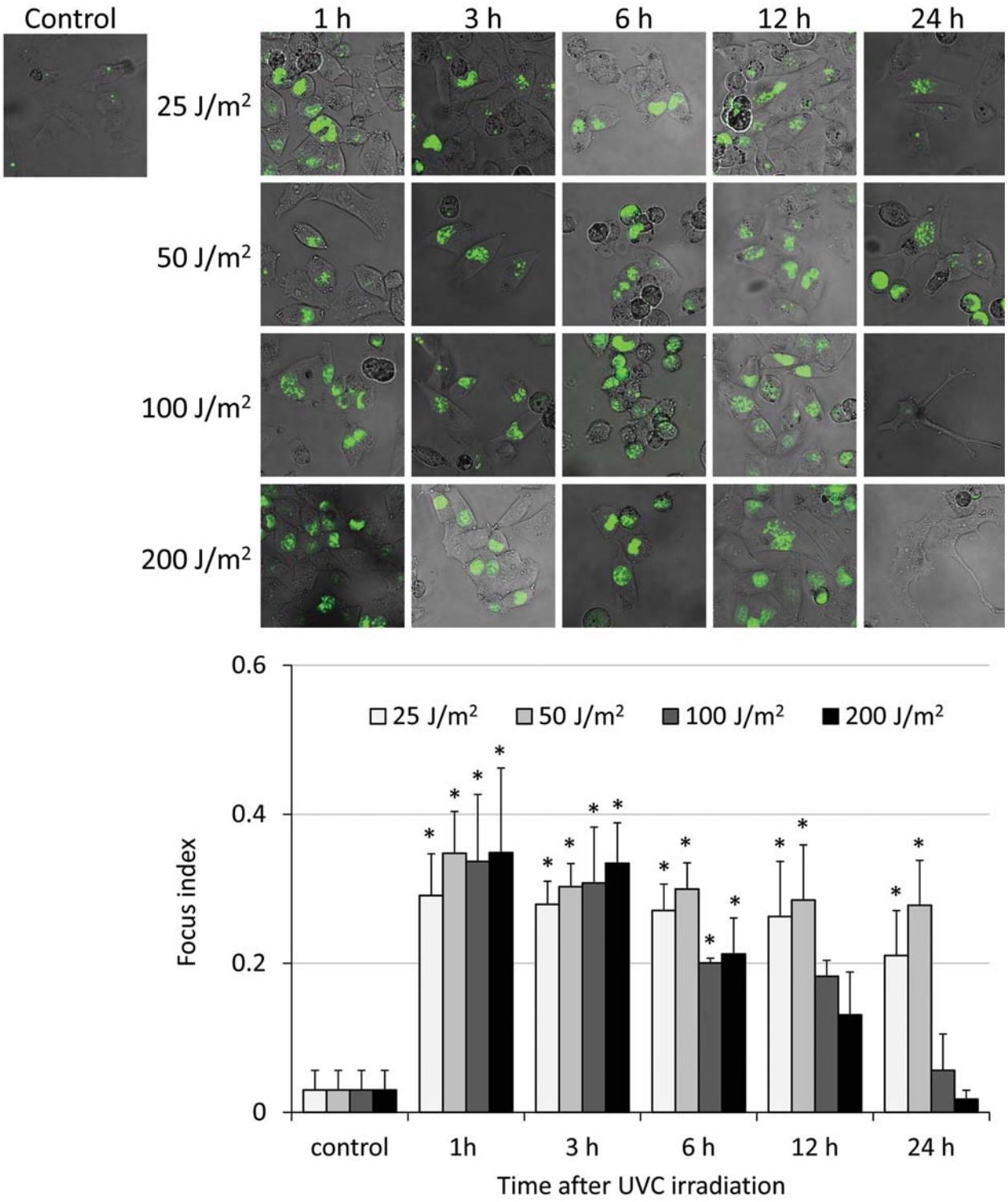

Figure 2. *UVC dose response for GFP-53BP1 focus formation in MiaPaCa2$^{Tet\text{-}On}$ GFP-53BP1 cells. MiaPaCa2$^{Tet\text{-}On}$ GFP-53BP1 cells were irradiated with 25, 50, 100, and 200 J/m^2 UVC. After incubation in a CO_2 incubator at 37˚C, GFP-53BP1 focus formation was observed. There was a small number of foci formed in control cells. There were large numbers of focus-positive cells at 1 and 3 h after UVC irradiation with no significant differences due to the doses of UVC irradiation. GFP-53BP1 focus formation continued for 24 h in the cells which were irradiated with low doses of UVC (25 and 50 J/m^2). In contrast, the cells irradiated with high doses of UVC (100 and 200 J/m^2) exhibited decreases of GFP-53BP1 focus formation and apoptotic changes at 6, 12 and 24 h after UVC irradiation. The experimental data are expressed as the mean ± SD. Statistical analysis was performed using ANOVA. *p<0.05 compared with control.*

The effect of UVC irradiation was also previously investigated by our laboratory on a model of brain cancer and a model of experimental brain metastasis (4). The U87 human glioma cell line was used for the brain cancer model, and LLC was used for the experimental brain metastasis model. Both cancer cell types were labeled with GFP in the nucleus and RFP in the cytoplasm. A craniotomy open window was used to image single cancer cells in the brain. This double labeling of the cancer cells with GFP and RFP enabled apoptosis of single cells to be imaged at the subcellular level through the craniotomy open window. UVC irradiation, beamed through the craniotomy open window, induced apoptosis of the cancer cells on the brain as imaged in live mice. UVC irradiation was more effective on LLC and significantly extended the survival of the mice. In contrast, the U87 glioma was relatively resistant to UVC irradiation. We also observed that the expression of fluorescent proteins in cancer cells can enhance UV killing (5).

In the present study, focus formation by the DDR protein 53BP1 fused to GFP was imaged after UV irradiation in MiaPaCa-2 human pancreatic cancer cells in order to further understand the mechanism of cancer-cell response to UV irradiation.

Materials and Methods

Cell culture, gene vectors and transfection. GFP fused to the human 53BP1 binding domain was cloned into the pLVX-Tight-Puro lentivival vector (Clontech Laboratories, Mountain View, CA, USA) (1). The vector was then transduced into the MiaPaCa-2 Tet-On Advanced cell line (Clontech) and cultured in high-glucose (DMEM) (Life Technologies, Grand Island, NY, USA) with 10% Tet system-approved fetal bovine serum (Clonetech). MiaPaCa-2 Tet-On Advanced is certified by Clontech as being derived from MiaPaCa-2 (American Type Culture Collection, Manassas, VA, USA) by viral transduction. After induction for 48 h with 1 μg/ml doxycycline (Sigma-Aldrich, St. Louis, MO, USA), GFP-positive cells were sorted to establish a stable MiaPaCa-2$^{\text{Tet-On}}$ GFP-53BP1 cell line.

UV irradiation. For UV irradiation, the cells were cultured on 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-3 UV transilluminator (UVP, LLC, Upland, CA, USA), 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).

Time-lapse imaging of GFP-53BP1 focus formation induced by UVC irradiation in MiaPaCa-2$^{\text{Tet-On}}$ GFP-53BP1 cells. To determine the time-course of GFP-53BP1 focus formation after UVC irradiation, time-lapse imaging of MiaPaCa-2$^{\text{Tet-On}}$ GFP-53BP1 cells was performed. MiaPaCa-2$^{\text{Tet-On}}$ GFP-53BP1 cells were cultured in a 35 mm dish for 48 h. Then, the cells were irradiated by 300 J/m^2 UVC. GFP-53BP1 foci were then imaged with an FV1000 confocal laser microscope (Olympus Corp., Tokyo, Japan). 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, Planegg, Germany).

Time course of GFP-53BP1 focus induction by various doses of UVC irradiaton in MiaPaCa-2$^{\text{Tet-On}}$ GFP-53BP1 cells. To determine if UVC-induced GFP-53BP1 focus formation is dose dependent, MiaPaCa-2$^{\text{Tet-On}}$ GFP-53BP1 cells were seeded into 35 mm dishes and treated with 1 μg/ml doxycycline for 48 h. The cells were then irradiated with 25, 50, 100, and 200 J/m^2 UVC. GFP-53BP1 focus formation was observed with an FV1000 confocal laser microscope and counted.

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 of 0.05 or less were considered statistically significant.

Results

Time course of GFP-53BP1 focus formation after UVC irradiation of MiaPaCa2$^{\text{Tet-On}}$ GFP-53BP1 cells. MiaPaCa-2$^{\text{Tet-On}}$ GFP-53BP1 cells were seeded into 35 mm dishes and treated with 1 μg/ml doxycycline for 48 h. The cells were then irradiated with 300 J/m^2 UVC GFP-53BP1. Focus formation was observed with the FV1000 confocal microscope. There were only a few foci before UVC irradiation. Focus formation was visible within 15 min after UVC irradiation (Figure 1). GFP-53BP1 foci remained visible for at least three hours after UVC irradiation.

Dose response of UV-induced GFP-53BP1 focus formation in MiaPaCa2$^{\text{Tet-On}}$ GFP-53BP1 cells. Following induction with doxycycline, untreated MiaPaCa2$^{\text{Tet-On}}$ GFP-53BP1 cells displayed only rare foci (Figure 2). The cells irradiated with 25-50 J/m^2 doses of UVC had an increased number of foci remaining for at least 24 hours. 100-200 J/m^2 UVC reduced the cell number at 24 hours, probably due to apoptotic cell death. Cells which had 5 or more foci were considered as positive cells. The focus index was defined as the percentage of positive cells.

Discussion

Our previous studies have shown the efficacy of UV light on cancer cells expressing fluorescent proteins. The fluorescent protein-expressing cells were very sensitive to UV light. As little as 25 J/m^2 UVC irradiation killed 70% of fluorescent protein-expressing cancer cells. UVC-killing of cancer cells was enhanced by fluorescent protein expression compared to non-expressing cells (5). UVC exposure also suppressed cancer cell growth in a nude mouse model of minimal residual cancer (MRC). No apparent side effects of UVC exposure were observed (2). 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 (7) was determined. The GFP-expressing melanoma and RFP-expressing blood vessels in the untreated 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 (3). UVC also killed cancer cells growing on the brain (4).

Ionizing radiation (IR) induces double-strand DNA breaks (DSB) in cancer cells. Although UVC light also induces DNA damage (6) there is little information on the behavior of DNA damage-response (DDR) proteins after UV irradiation of cancer cells.

In the current study, real-time imaging demonstrated GFP-53BP1 focus formation occurred within 15 minutes after UVC irradiation of human pancreatic cancer cells. Thus, GFP-53BP1 is a useful marker of early response to UVC irradiation and can be exploited to increase the efficacy of UV killing of cancer cells.

An important possible clinical application of UV-light cancer treatment would be to sterilize the surgical bed after fluorescence-guided surgery of fluorescent protein-expressing cells. In this regard, OBP-401, a telomerase-dependent, replication-competent adenovirus expressing GFP was used to label tumors in situ. GFP is a powerful label for *in vivo* imaging (8-12). HCT-116, in a model of intraperitoneal disseminated human colon cancer, was labeled by OBP-40 virus injection into the peritoneal cavity of nude mice. Only the malignant tissue fluoresced brightly. Fluorescence-guided surgery enabled resection of tumor nodules labeled with GFP by OBP-401 (13).

However, technical problems with resecting all cancer cells still remain, even with fluorescence-guided surgery. Recurrent tumor nodules after fluorescence-guided surgery brightly expressed GFP, indicated that initial OBP-401 GFP labeling of peritoneal disease was genetically stable. In situ tumor labeling with a genetic reporter has important advantages over antibody and other non-genetic labeling of tumors, since any residual disease remains labeled during recurrence and can be further resected under fluorescence guidance. UV light therapy would have an important application to kill remaining fluorescent cancer cells after fluorescence-guided surgery (14-16).

Conflicts of Interest

None of the Authors have a conflict of interest regarding this study.

Acknowledgements

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