

## Fluorescent Proteins Enhance UVC PDT of Cancer Cells

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**Abstract.** Cancer cells, with and without fluorescent protein expression, were irradiated with various doses of UVC (100, 400, and 600 J/m<sup>2</sup>). Dual-color Lewis lung carcinoma cells (LLC) and U87 human glioma cells, expressing GFP in the nucleus and RFP in the cytoplasm and non-colored LLC and U87 cells were cultured in 96-well plates. Eight hours after seeding, the cells were irradiated with the various doses of UVC. The resulting cell number was determined after 24 hours. Compared to non-colored LLC cells, the number of dual-color LLC cells decreased significantly due to UVC irradiation with 100 J/m<sup>2</sup> ( $p=0.003$ ). Although there was no significant difference in the number of dual-color and non-colored U87 cells after 100 J/m<sup>2</sup> UVC irradiation ( $p=0.852$ ), the number of dual-color U87 cells decreased significantly with respect to non-colored cells due to UVC irradiation with 400 J/m<sup>2</sup> and 600 J/m<sup>2</sup> ( $p=0.011$  and  $p=0.009$ , respectively). Thus, both dual-color LLC and dual-color U87 cells were more sensitive to UVC light than non-colored LLC and U87 cells. These results suggest that the expression of fluorescent proteins in cancer cells can enhance photodynamic therapy (PDT) using UVC and possibly with other wavelengths of light as well.

Photodynamic therapy (PDT) has been shown to be effective for certain cancer types (1). UV light has been used for the phototherapy of cutaneous malignancies. Psoralen plus UVA (PUVA) and narrowband UVB were found to be effective (2-4). However, the effect of UV light on cancer cells is not well-understood (5-7). UV light has been mainly used for skin cancer, since short wavelength light does not penetrate deeply through the skin (8).

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Our laboratory pioneered *in vivo* imaging with fluorescent proteins (9-11). We have also developed dual-color cancer cells, in which red fluorescent protein (RFP) is expressed in the cytoplasm and green fluorescent protein (GFP), linked to histone H2B, is expressed in the nucleus. Nuclear GFP expression enables for visualization of nuclear dynamics such as apoptosis, whereas simultaneous cytoplasmic RFP expression enables visualization of nuclear-cytoplasmic ratios as well as cytoplasmic and nuclear shape changes (12-15).

We previously investigated the cell-killing efficacy of UV light on various cancer cell lines expressing GFP in the nucleus and RFP in the cytoplasm *in vitro* and *in vivo* (8).

The efficacy of fluorescence-guided ultraviolet C (UVC) irradiation on the growth of murine melanoma expressing GFP in the ear of RFP mice was previously determined and found to inhibit melanoma growth and also damaged blood vessels in the tumor (16).

The present study directly compares the cell-killing efficacy of UVC on cancer cells with and without fluorescent protein expression in order to determine if fluorescent proteins enhance PDT.

### Materials and Methods

**Cells.** To establish Lewis lung carcinoma (LLC) or U87 human glioma (U87) cells expressing GFP in the nucleus and RFP in the cytoplasm, the cells were transfected with retroviral RFP and H2B-GFP vectors as previously described (12, 14, 17, 18). Dual-color LLC and U87 cells were maintained in DMEM medium (Hyclone Laboratories, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories). The cells were incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. The cells were collected after trypsinization and stained with trypan blue (Sigma-Aldrich, St. Louis, MO, USA). Only viable cells were counted with a hemocytometer (Hausser Scientific, Horsham, PA, USA).

**Sensitivity of dual-color and non-colored cancer cells to UVC light *in vitro*.** To determine if UV-induced cancer cell death is

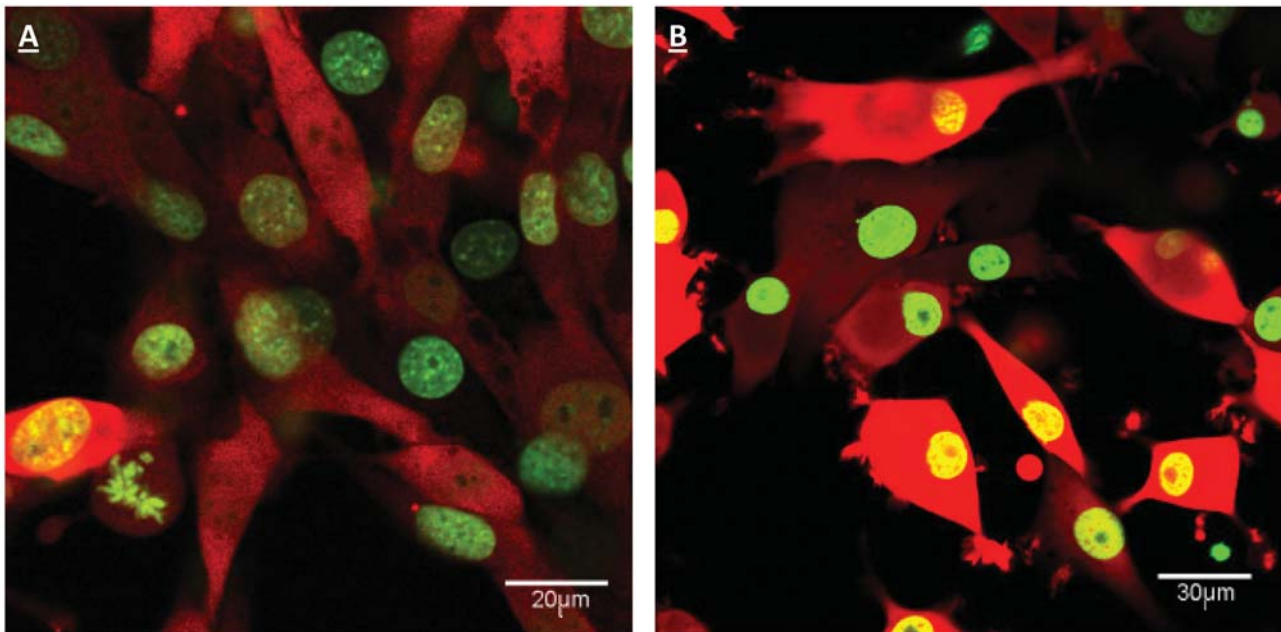


Figure 1. A) Dual-color mouse LLC cells. B) Dual-color human U87 glioma cells. The dual-color cancer cells express GFP, linked to histone H2B, in the nucleus and RFP in the cytoplasm. Cells were cultured *in vitro*, as described in the Material and Methods. Cells were observed under confocal microscopy using the FV1000 (Olympus Corp., Tokyo, Japan).

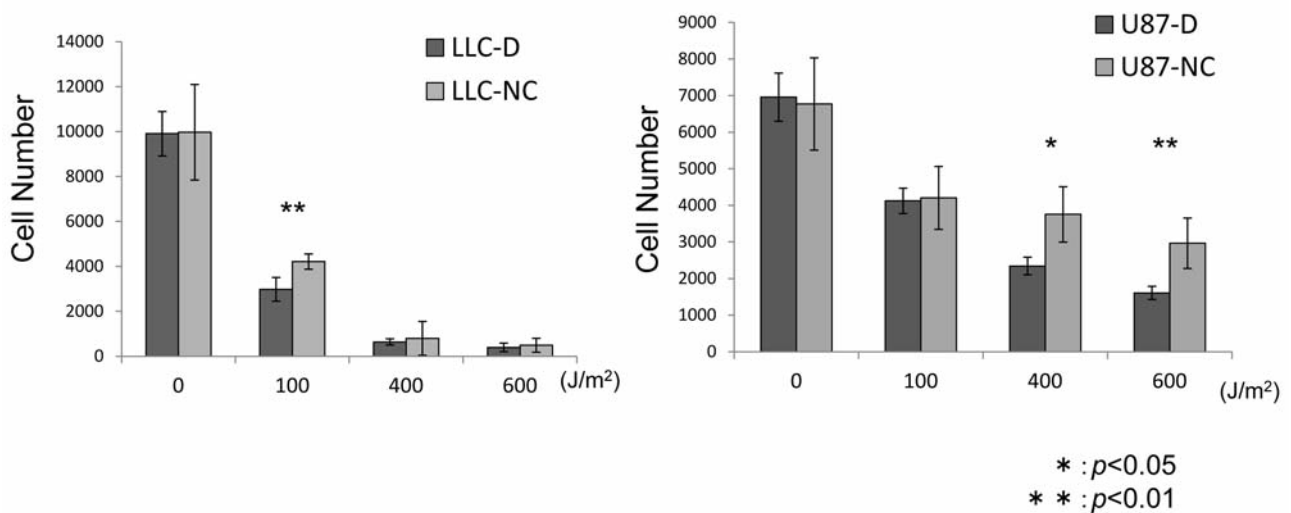


Figure 2. Efficacy of UVC irradiation on fluorescent protein-expressing and non-colored cancer cell lines *in vitro*. UVC was irradiated at various doses (100, 400, and 600 J/m²). Dual-color LLC (LLC-D) cells and non-colored LLC (LLC-NC) cells (8,000/well) were plated in 100 µl cell culture medium per well in 96-well plates. Eight hours after seeding, the cells were irradiated with various doses of UVC (0-600 J/m²). The cell number was determined after 24 hours. Compared to non-colored LLC cells, the number of dual-color LLC cells decreased significantly due to UVC irradiation with 100 J/m² ( $p=0.003$ ). Although there was no significant difference in the number of dual-color and non-colored U87 cells after 100 J/m² UVC irradiation ( $p=0.852$ ), the number of dual-color U87 cells (U87-D) decreased significantly with respect to non-colored U87 cells (U87-NC) due to UVC irradiation with 400 J/m² and 600 J/m² ( $p=0.011$  and  $p=0.009$ , respectively). Both dual-color LLC and dual-color U87 cells were more sensitive to UVC light than non-colored LLC and U87 cells. Five wells were used for each cell line and each UV dose. The experimental data are expressed as the mean  $\pm$  SD. Statistical analysis was performed using the Student's *t*-test.

enhanced by the expression of fluorescent proteins, dual-color LLC cells and U87 glioma and non-colored LLC cells and dual-color U87 glioma cells and non-color U87 glioma cells (8000/well) were plated in 100  $\mu$ l medium per well in 96-well plates. Eight hours after seeding, the cells were irradiated with various doses of UVC (0-600 J/m<sup>2</sup>) (8). Cell number was measured after 24 hr with the CellTiter96 Aqueous Non-radioactive Assay (Promega Corp., Madison, WI, USA). Five wells were used for each cell line and each UV dose.

**Statistical analysis.** The experimental data are expressed as the mean  $\pm$  SD. Statistical analysis was performed using the Student's *t*-test or the Kruskal-Wallis test. A *p*-value of less than 0.05 indicated a significant difference.

## Results

Images of LLC and U87 cells expressing GFP in the nucleus and RFP in the cytoplasm are shown Figure 1A and B.

*In vitro* experiments indicated increased efficacy of UVC light on cancer cells labeled with fluorescent proteins (Figure 2). Compared to non-colored LLC cells, the number of dual-color LLC cells decreased significantly due to UVC irradiation with 100 J/m<sup>2</sup> (*p*=0.003).

Although there was no significant difference in the number of dual-color and non-colored U87 cells after 100 J/m<sup>2</sup> UVC irradiation (*p*=0.852), the number of dual-color U87 cells decreased significantly due to UVC irradiation with 400 J/m<sup>2</sup> and 600 J/m<sup>2</sup> (*p*=0.011 and *p*=0.009, compared to non-colored cells, respectively).

## Discussion

Fluorescent-protein-expressing dual-color cancer cells were more sensitive to UVC light than non-colored cancer cells (Figure 2). Therefore, UVC light may be a powerful tool for treatment of cancer cells expressing fluorescent reporters which now can be introduced selectively to cancer cells *in vivo* (19, 20).

UVC light does not deeply penetrate tissue. UVC light, however, could be of potential use to sterilize the surgical bed after tumor resection. Fluorescent proteins are also excited at longer wavelengths. GFP is, for example, excited at 484 nm and RFP at 563 nm (21). Our results, thus, open up the possibility of PDT using fluorescent proteins at longer wavelengths to enhance PDT. Since longer wavelength light penetrates tissue more deeply, PDT of cancer cells with fluorescent protein expression could be used for more deep-seated cancers.

## Conflicts of Interest

None of the Authors has any conflicts of interest regarding this study.

## Acknowledgements

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