Focal Points

Application Note FP-156

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Real-Time Imaging of Nuclear-Cytoplasmic Dynamics in UV Light Killing of Cancer Cells Expressing Fluorescent Proteins

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Introduction

Our laboratory pioneered dual-color cancer cells, in which red fluorescent protein (RFP) is expressed in the cytoplasm and green fluorescent protein (GFP) in the nucleus. Total cellular dynamics can be visualized in the living dual-color cells in real time. In this study, we investigated the cancer-cell-killing efficacy of UV light using the dual-color cancer cells in vitro and in vivo.

For in vitro experiments, a Benchtop 3UV™ transilluminator (UVP), 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; was used. For in vivo experiments, nude mice were seeded with RFP expressing cancer cells subcutaneously. UVC treatment was given 48 hours later. An ***iBox*® *Scientia Small Animal Imaging System* (UVP)** was used to measure the size of the fluorescent tumor every five days to evaluate the efficacy of the treatment.

Methods and Results

In-Vitro Experiments

After exposure to various doses of UVA, UVB, or UVC, apoptotic, necrotic and viable cells were quantitated under fluorescence microscopy using dual-color 143B human osteosarcoma cells (143B). UV-induced cancer cell death was wave-length and dose dependent. After UVA exposure, most cells were viable even if the UV dose was increased up to 200 J/m2.

In the UVB group, cell death began to appear when irradiated at 50 J/m2. For UVC, the rate of cell killing was proportional to increased UVC irradiation. 25 J/m2 UVC irradiation killed over 40% of the 143B dual-color cells. However, the rate of cell killing plateaued at 100 J/m2. We also tested 50J/m2 UVC and 100J/m2 of UVB on four types of dual-color cancer cell lines. UV-induced cancer cell death varied among the cell lines. Cell death began about 4 hours after irradiation and continued until 10 hours after irradiation. Real-time movies were made of cells undergoing UV-induced cell death. Most cells died via apoptosis and only a small portion of them died via necrosis. We will develop UV irradiation for treatment of fluorescent-protein-expressing minimal residual cancer remaining after resection.

Figure 1. Dose and wavelength dependency of UV induced cell death and morphological changes.

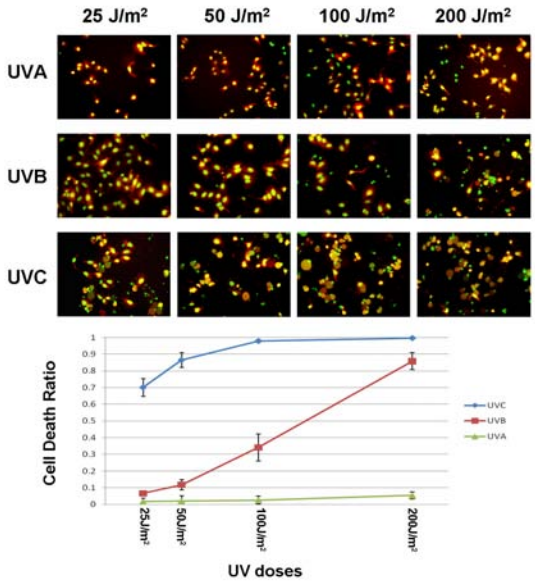
143B dual-color cells were irradiated with 25, 50, 100, and 200 J/m2 of UVA, UVB, and UVC. Cells were then incubated for 24

iBox Scientia Small Animal Imaging System

UVA irradiation of 143B dual-color cells. Most cells were viable without change of morphology. In a few cells, cytoplasm was shed without change of nuclear shape.

UVB irradiation of 143B dual-color cells. Apoptotic cells began to appear among cells irradiated at 50 J/m2 and the frequency of apoptotic cells gradually increased with higher UV exposure. Nuclear condensation and fragmentation were observed.

Figure 1. **Dose & wave-length dependency of UV-induced cell death in 143B dual cells**



UVC irradiation of 143B dual-color cells. UVC irradiation induced cancer cell death at the highest frequency. The frequency of apoptotic cells reached a plateau at 100 J/m2. Cell shrinkage, nuclear condensation, and fragmentation were observed.

These results indicated that UV-induced cancer cell death was wave-length and dose dependent. (Data are the averages of eight chamber and *bars* show the S.D. values.)

Figure 2. Cell line dependency of UV-induced cell death.

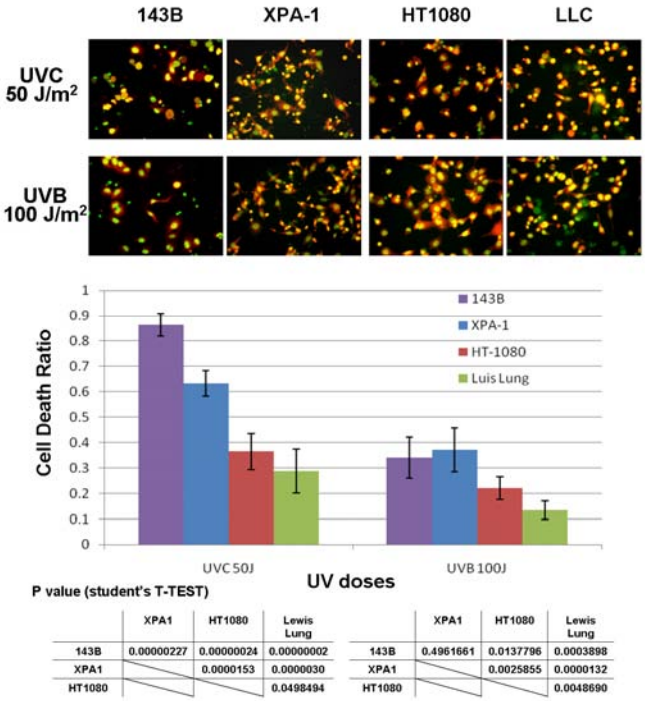
50 J/m2 UVC or 100 J/m2 UVB were irradiated on four different types of cancer cell lines, 143B osteosarcoma, HT1080 fibrosarcoma, XPA-1 pancreatic cancer, and Lewis lung carcinoma (LLC). The number of apoptotic cells and necrotic cells and viable cells were counted under fluorescence microscopy after 24 hours subsequent culture.

50 J/m2 UVC. 143B cells were the most sensitive to UVC light. The morphology of apoptotic cells varied slightly among cell lines.

100 J/m2 UVB. The cell lines had the same relative sensitivity to UVB as UVC.

Data are the averages of eight chamber and *bars* show the S.D. values. Statistical differences were analyzed using the Student's *t*-test. This graph indicates that the sensitivity to UV irradiation varied among cell lines.

Figure 2. Cell line dependency of UV-induced cancer cell death



P value (student's T-TEST)

	XPA1	HT1080	Lewis Lung
143B	0.00000227	0.00000024	0.00000002
XPA1		0.0000153	0.0000030
HT1080			0.0498494

	XPA1	HT1080	Lewis Lung
143B	0.4961661	0.0137796	0.0003898
XPA1		0.0025855	0.0000132
HT1080			0.0048690

In Vivo Experiments

Experimental protocol. Diagram of MRC model and UVC treatment. After anesthesia, 5 mm rectangular incisions were made bilaterally on the flanks of nude mice. LLC cells (5×105) in 10 µl PBS were then seeded, and the incisions were closed. 48 hours later, the bilateral incisions were reopened, and 100 J/m2 UVC was irradiated only to the right-flank tumor for 180 seconds with the customized UVC pen light.

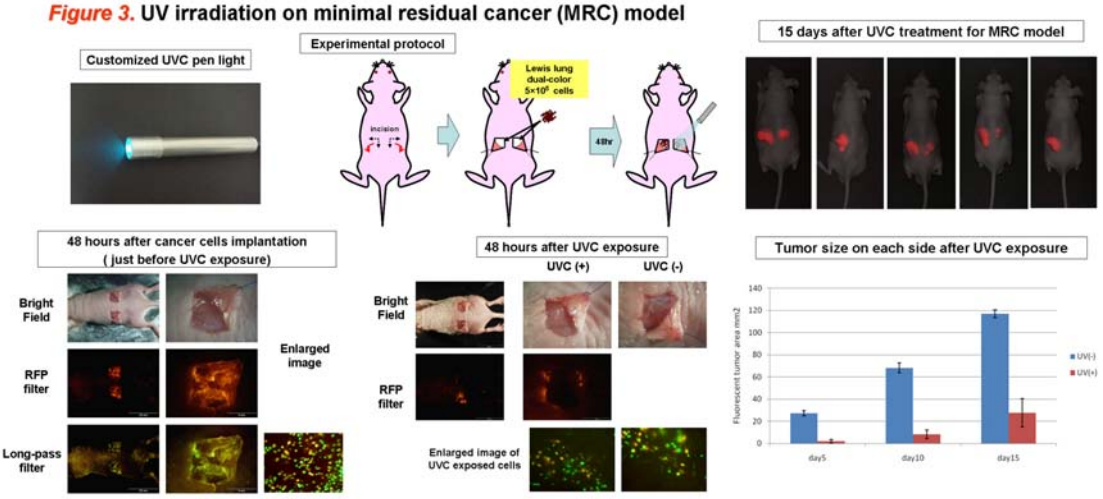
48hrs after cells implantation. Fluorescence images demonstrated many cancer cells still remained in the bilateral surgical field.

48hrs after UVC exposure. Images showed fewer cancer cells in the irradiated flank than in the untreated flank. Enlarged images show that many cancer cells appeared apoptotic. The cellular level images were obtained with the OV100 Small Animal Imaging System (Olympus Corp.).

Non-invasive imaging of efficacy of UVC treatment of MRC. After UVC irradiation of the MRC model, the mice were imaged once every 5 days, using the *iBox Scientia Small Animal Imaging System* (UVP). Merged images of bright field and RFP fluorescence at day 15 were shown. In all mice, the right-side tumors, which were irradiated with UVC, were smaller than the left-side tumors. In mouse 5, UVC exposure completely irradicated the tumor formation.

Tumor size of each side after UVC treatment. Fluorescent area (mm2) of residual tumors at days 5, 10, and 15. The experimental data were expressed as the mean ± SE of 5 mice. The statistical difference in size between UV-treated and untreated tumors was analyzed using the Student's t-test. Average tumor size of the UV-treated group was significantly smaller than the untreated group.

Figure 3. UV irradiation on minimal residual cancer (MRC) model



As discussed in Figure 3, after UVC irradiation of the MRC model, the mice were imaged once every 5 days, using the *iBox Scientia Small Animal Imaging System.*

Conclusion

UV light could induce apoptosis in cancer cells. The data in the present report indicates that shorter wavelength UV light was more effective killing of cancer cells in vitro. Therefore, we utilized UVC for the treatment of MRC in mice. Although UVC light does not deeply penetrate tissue, we assumed that it could be effective in preventing tumor formation from MRC and minimize the side effect of UV treatment for normal tissue.

The present study showed that UVC irradiation of MRC inhibited subsequent tumor formation, without any obvious side effects. This study opens up the possibility of UVC treatment for MRC after surgical resection. This approach has promising clinical potential.

References

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