Experimental Methods

Cell culture

B16-F10 (*Mus musculus* skin melanoma), NCI-H460 (human non-small cell lung cancer cells), MDA-MB-231 (metastatic human breast cancer cell line), and 4T1 (*Mus musculus* mammary breast cancer) cell lines were purchased from the Korean Cell Line Bank. B16F10 cells were maintained in Dulbecco's modified Eagle's medium (Hyclone); other cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal bovine serum (FBS; Hyclone) in a humidified atmosphere containing 5% CO_2 at 37 °C.

Measurement of fluorescence *in vitro*

One million of NCI-H460 and NCI-H460-GFP cell lines were cultured in RPMI-1640 medium supplemented with 10% FBS in 75-cm^2 Cell culture dish. After 12 h, media was changed with FBS free RPMI media and various concentrations (0 μM, 5 μM, 10 μM, 50 μM) of RB were added in experimental cell culture flasks. After 4-h incubation for intracellular accumulation of RB, cells were centrifuged and collected in conical tube. pellets of each cell groups were dispersed into 1-ml PBS. Emission spectrum of each cell groups was measured using spectrofluorometer (JASCO Inc.). Then, emission spectrum of RB non-treated and various concentrations of RB treated NCI-H460 cells and NCI-H460-GFP cells were measured upon 473 nm excitation. Measurements of each group were pentaplicated. FRET efficiency was calculated by previously reported methods.[1]

$$E = 1 - \frac{F(DA)}{F(D)}$$

E is FRET efficiency. F(DA) and F(D) mean fluorescence intensity of donor (GFP) in the presence of acceptor (RB) and absence of acceptor (RB), respectively.

In vitro PDT experiment

The photodestruction of GFP non-expressing and GFP expressing cells after irradiation with blue light was assessed by MTT assay in the presence or absence of RB. RB was diluted with DMEM containing 10% FBS to achieve a series of equivalent concentrations. The concentrations of RB were 6.25, 12.5, 25, 50, and 100 μM. GFP non-expressing and GFP expressing cells were pre-cultured for 24 h in 96-well, black, and clear bottom plates at a density of 2×10^4 cells per well. The cells were incubated with RB for 4 h. For the untreated control group, fresh culture medium without photosensitizers was added to the wells. The cells were washed twice with PBS buffer. For the PDT-treated groups, the cells were irradiated with blue laser light (473 nm; irradiation dose rate = 80 mW/cm^2) for 60 s. The toxicity of pure RB to GFP expressing and GFP non-expressing cells was evaluated in the dark. After irradiation, fresh culture medium was added to the wells and the cells were incubated for another 24 h. Then, the medium was assessed by MTT assay. Briefly, 150 μL of the solubilization solution and the stop solution was added, followed by incubation at 37 °C for 4 h. The absorbance of the reaction solution was measured at 570 nm. Cell viability was calculated using the following formula: $(OD_{treated}/OD_{control}) \times 100\%$; three independent experiments were performed. To reduce the bias among kinds of cells, four kinds of non-GFP expressed cell lines like 4T1 mammary carcinoma, B16-F10 melanoma cell lines, MDA-MB-231 breast carcinoma and NCI-H460 non-small-cell lung carcinoma cell lines and were used. GFP expressed cells of each cell line were also used. Each of cell lines were incubated in RB of 12.5 μM. The MTT experiments were performed in same method as mentioned above.

After the cells were seeded into 24-well plates at a density of 5×10^4 cells per well, they were treated with photosensitizers and blue light as discussed above. The media were prepared separately. Briefly, 100 μL of the supernatant of the cultured cells was transferred from each

well to the corresponding wells of a new plate, and 100 μL of the reaction solution was added to each well. Plates were incubated with gentle shaking on an orbital shaker for 30 min at room temperature. The absorbance was read at 490 nm by using a plate reader.

Tumor-implanted animal experiments

B16F10 and GFP-B16-F0 melanoma cells were harvested using a cell lifter and re-suspended in serum-free DMEM. About 2×10^6/100 μL of B16-F0 and GFP-B16-F0 melanoma cells were subcutaneously injected into both flanks of female C57BL/6 mice. After 10~15 days, when the mean tumor diameter reached a minimum of 0.3 cm, the mice were divided into groups of 4, based on their diameter. Then, these mice were randomized into 4 groups (n = 5 per group). Animals in the control group received only 0.9% saline instead of chemicals. Experimental groups were intravenously injected with RB (363 mg/mL, 53 nM/mL), followed by irradiation of the tumor region with blue light (2 min) after 4 h of injection, twice a week. The body weight of these mice was determined, and tumor volumes were estimated twice a week. After 3 weeks, the mice were euthanized, and the tumors were removed and fixed in 10% neutral buffered formalin. Images were captured the next day. For GFP^+ and GFP^- H460 cells, a similar procedure was performed. About 2×10^6/100 μL of cells were subcutaneously injected into both flanks. All animal experiments were approved by the Institutional Animal Care and Use Committee at Wonkwang University (WKU 15-136) and were performed in compliance with the institutional guidelines.

References

1. Y. Zhang and T.-H. Wang, *Theranostics*, 2012, 2, 631.

Detailed Figure legends

Fig. 1. Concept of experiment using energy transfer between GFP and RB

(A and B) Absorbance and emission spectra of GFP and RB; GFP, Green Fluorescent Protein; RB, Rose Bengal; Ab, Absorption; Em, Emission; Ex, Excitation; a.u, arbitrary unit (C) The scheme of selective cell death induced by blue laser light required for RB activation.

Fig. 2. Emission spectrum of NCI-H460 cells according to RB concentration upon excitation at 473 nm

(A) Emission spectrum of NCI-H460-GFP cells treated with different RB concentration and that of NCI-H460 cells treated with 10 μM RB after excitation at 473 nm. (B) Comparison of emission spectra of NCI-H460-GFP and NCI-H460 cells treated with 10 μM RB upon excitation at 473 nm; GFP, Green Fluorescent Protein; RB, Rose Bengal; a.u, arbitrary unit.

Fig. 3. Measurement of cytotoxicity against GFP expressing and GFP non-expressing cells *in vitro*

(A) The MTT assay was performed to measure the selective death of GFP expressing and GFP non-expressing cells after laser irradiation; PS, Photosensitizers; Ce6, Chlorin e6; PPIX, Protoporphyrin IX; RB, Rose Bengal; grey, GFP non-expressing cells; green, GFP expressing cells; scale bar, 50 μm. (B) Determination of cytotoxicity by using gradient bitmap graphics at different photosensitizer concentrations and exposure times; upper, GFP non-expressing cells;

lower, GFP expressing cells. (C) Measurement of the difference in cytotoxicity against the four GFP expressing cancer cell types, namely, MDA-MB-231, 4T1, H460, and B16-F10 cells, by performing the MTT assay; ns, no significance; ***$P < 0.001$. (D) Change in cytotoxicity according to the concentration of *GFP* transfected; EV, empty vehicle.

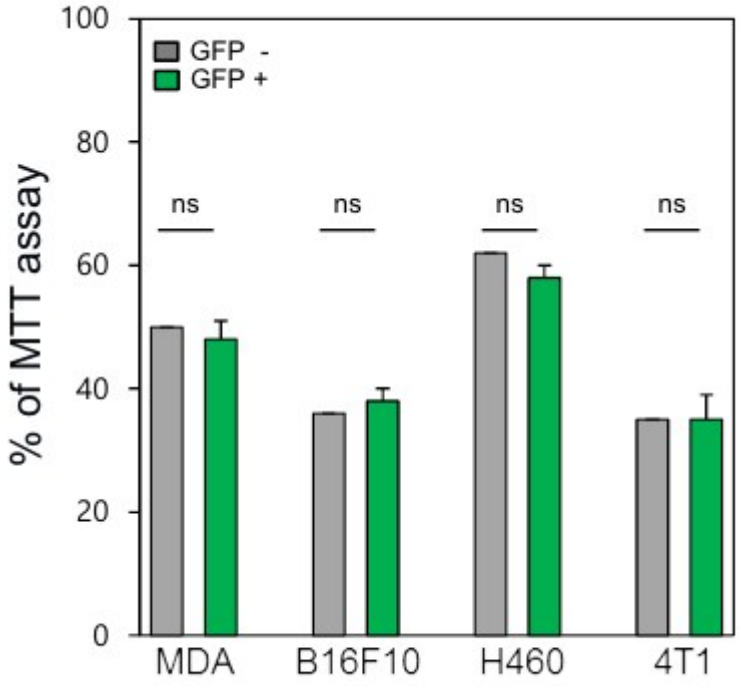
Fig. 4. Selective killing of GFP expressing cells in the GFP expressing tumour cell-implanted mouse model

(A) Irradiation of tumours under the skin flap at the site of tumour cell implantation by using 488-nm laser light. (B) Fluorescent confocal microscopic images of GFP expressing cells before and after 2 rounds of irradiation. (C and D) Areas of GFP^+ cell implantation were analysed using Image J 2.0 (C, B16F10 cells; D, H460 cells); a.u, arbitrary unit; scale bar, 100 μm; ***$P < 0.001$.

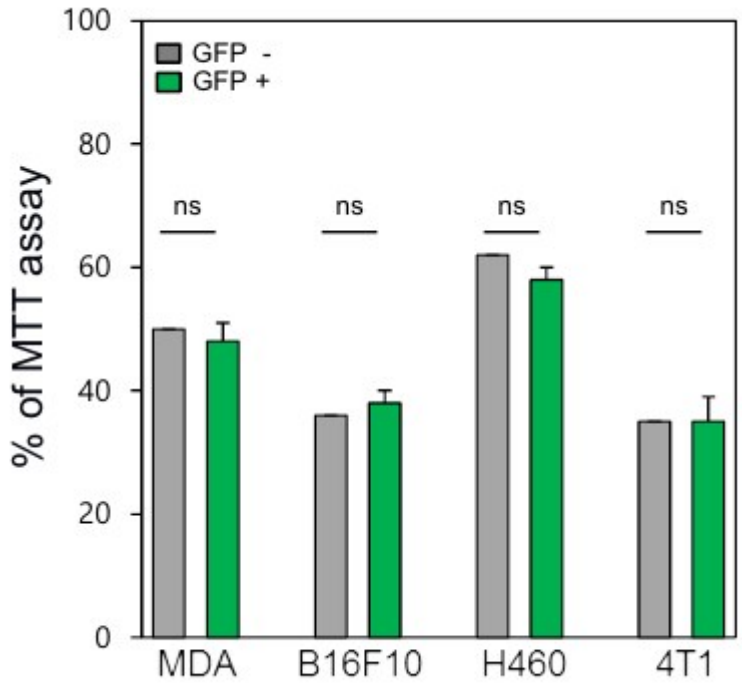
Fig. 5. PDT based on energy transfer in the GFP expressing cells-implanted mouse model

(A) Monitoring tumour growth from implanted GFP non-expressing or GFP expressing B16F10 cells comparing to other treatment combinations; *$P < 0.05$ and **$P < 0.01$. (B) Representative isolated tumours after various treatment combinations; scale bar, 1 cm. (C) Cell death was observed in the wide region beneath the tumour surface. The area containing dead cells was immunostained with anti-Bax antibody; scale bar, 200 μm. Expression level or secretion of (D) caspase-3 and (E) TNF-α was evaluated by performing immunohistochemical analysis; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$; scale bar, 50 μm.

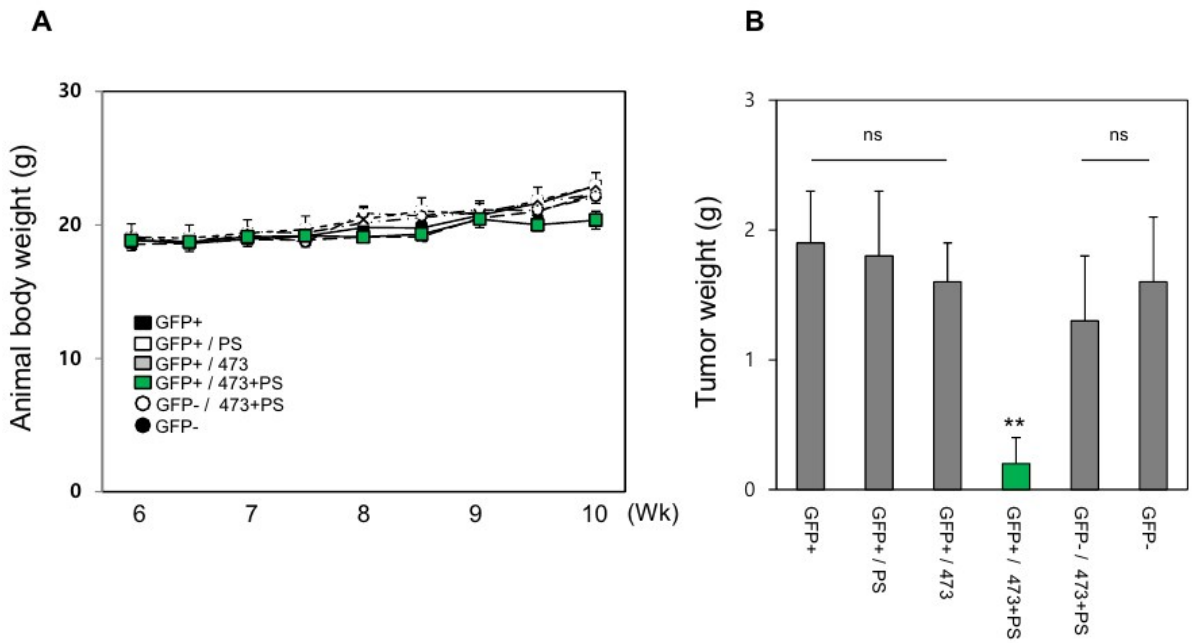
Supplementary Figures and Figure legends



Supplementary Figure 1. MTT assay to check differences of cytotoxicities between GFP expressing cancer cells and GFP non-expressing cancer cells with the combinatory treatment of Ce6 and 650nm red laser.



Supplementary Figure 2. Changes in fluorescence according to *GFP* DNA transfection amount. In 35mm dishes, different DNA amount was transfected into Hela cells. 24h after transfection, GFP intensity was observed with fluorescence microscopy. Scale bar, 20 μm



Supplementary Figure 3. In Fig. 5, B16-F10 and GFP-B16-F10 melanoma cells were subcutaneously injected into the flank of female C57BL/6 mice. Body weight and tumour volume of the mice were determined twice a week. Tumour volumes were estimated by using the formula: Tumour volume= length x width^2/2, where length represents the largest tumour diameter and width represents the perpendicular tumour diameter. After 3 weeks, the mice were euthanized and tumour volumes were estimated.