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Development of enzyme-activated photosensitizer based on intramolecular electron transfer

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ABSTRACT

Photosensitizers produce cytotoxic reactive oxygen species (ROS) upon light illumination, but it is difficult to ablate cells of a specific type (e.g., tumor cells) in the presence of other cell populations, because of the limited precision with which light illumination can be directed to small areas. Here, we report a strategy to achieve cell type-specific ablation by using an enzyme-activated off/on switch for oxidative stress induction. In the unactivated photosensitizer, induction of oxidative stress is quenched by intramolecular electron transfer. However, the target cells express an enzyme that hydrolyzes a substrate moiety of the photosensitizer and the activated photosensitizer induces oxidative stress. As proof of concept, we designed and synthesized a xanthene-based photosensitizer, TGI-βGal, whose oxidative stress induction ability is switched on following hydrolysis reaction with β-galactosidase, a widely used gene marker. TGI-βGal could selectively ablate *lacZ*-positive cells, whereas it showed no toxicity to *lacZ*-negative cells, upon light illumination.

Photosensitizers are chemical tools that produce reactive oxygen species (ROS) upon light illumination and are commonly used to cause light-induced cell killing, for example, in the treatment of cancer by means of photodynamic therapy (PDT).[1] Usually, the area of cell ablation is regulated by controlling the area of light illumination. However, it is difficult to distinguish and ablate a specific type of cell (e.g., tumor cells) in the presence of other cell populations without affecting the non-targeted cells, because the precision with which light illumination can be directed to small areas is limited. The development of photosensitizers that recognize specific types of cells and generate ROS only in them, enabling cell type-specific ablation, would be extremely useful.

Silencing a specific cell population is also an important and fundamental technique in biological research. For example, ablating specific cell populations of neural circuit in vitro and in vivo would allow us to understand how the activity in specific neuronal populations contributes to physiological processes and behavioral responses.[2,3] Light-triggered cell ablation using photosensitizers potentially has a great advantage, compared to other methods, including surgical excision and pharmacological techniques, because the timing and site of cell ablation can be precisely controlled by adjusting the timing and area of light illumination. A green fluorescent protein (GFP)-based photosensitizer (KillerRed)[4] was recently developed for this purpose. KillerRed can be introduced into and expressed in cells of interest, and light illumination can then be applied to specifically ablate those cells by means of light-induced ROS generation. However, the GFP-based photosensitizer has some drawbacks, including low efficiency of ROS generation compared with small molecule-based photosensitizers.

In this study, we report an approach to achieve cell type-specific ablation using a functional small-molecule-based photosensitizer with an off/on switching device. We focused on β-galactosidase,[5] a widely used gene expression marker, which can be easily introduced into a selected cell population, and utilized its hydrolytic activity as an off/on switch of ROS generation; that is, β-galactosidase serves to activate the photosensitizer, and thereby switches on local induction of oxidative stress by the photosensitizer inside the target cells. We have recently developed highly sensitive fluorescent probes for β-galactosidase (TG-βGal)[6] based on precise control of the photochemical properties of newly developed fluorescein derivatives (TokyoGreens, TGs) by photoinduced electron transfer (PeT) (Fig. 1a). The fluorescence of TG-βGal is efficiently quenched by electron transfer from the benzene moiety to the xanthene moiety and is restored by the change in the reduction potential of the xanthene moiety after hydrolysis by β-galactosidase. We hypothesized that if the xanthene moiety of TG-βGal could be converted to a photosensitizer moiety, it would behave similarly and could work as a β-galactosidase-dependent activatable photosensitizer, which would generate ROS only after activation via hydrolysis reaction by β-galactosidase. Such a strategy might have broad applicability.

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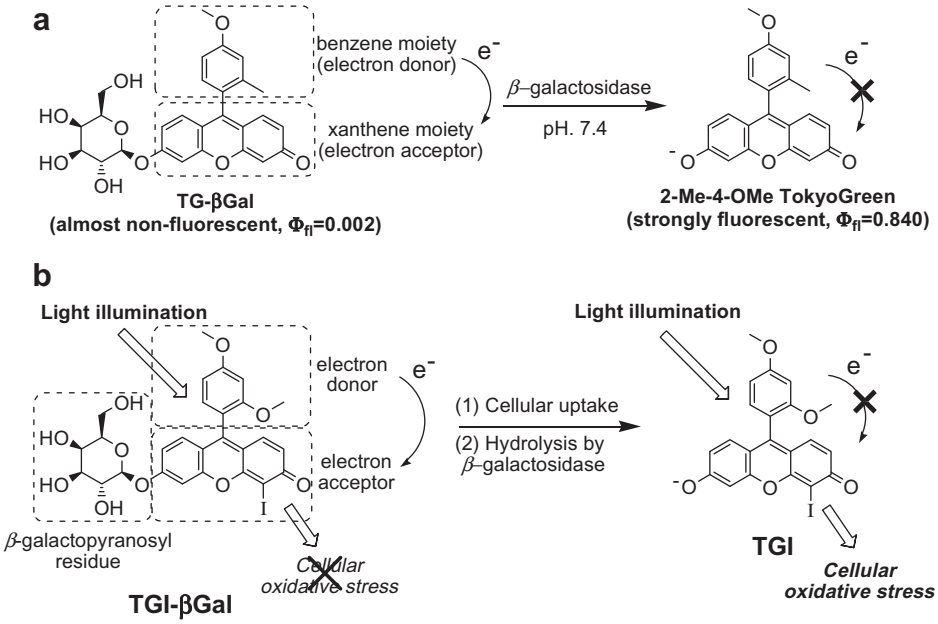
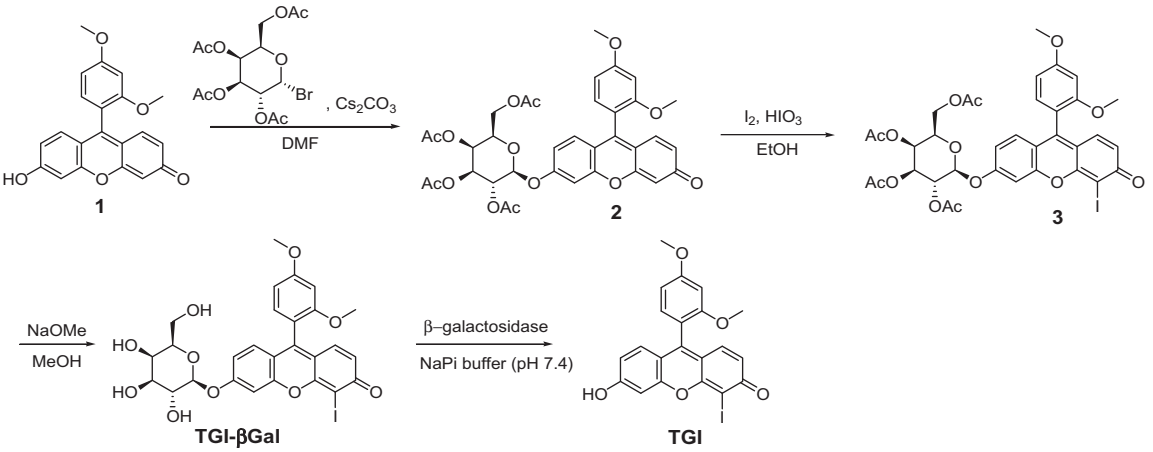


Figure 1. Development of β-galactosidase-dependent photosensitizer. (a) Reaction scheme of fluorescence probe for β-galactosidase (TG-βGal). (b) Reaction scheme of β-galactosidase-dependent photosensitizer (TGI-βGal).



Scheme 1.

To test this hypothesis, we designed and synthesized TGI-βGal (Fig. 1b and Scheme 1) by iodinating the xanthene moiety of TG-βGal in order to increase the singlet oxygen generation making use of the so-called internal heavy-atom effect,[7,8] and to increase the intracellular retention of the molecules by increasing their hydrophobicity. TGI-βGal was converted to TGI (Fig. 1b) by β-galactosidase without marked by-product generation (Supplementary Fig. 1), indicating that TGI-βGal is a good substrate for β-galactosidase. We then compared the ability of TGI-βGal to induce oxidative stress with that of TGI by following the appearance of the 351 nm absorbance band during oxidation of iodine ion (I^-) to I_3^-.[9,10] TGI-βGal was unable to oxidize I^-, whereas I^--oxidizing ability appeared in the presence of β-galactosidase (Fig. 2).

Table 1 summarizes the rate of I_3^- production at the initial stage (i.e., the slope), which corresponds to the efficiency of oxidative stress induction (ϕ_{oxi}). TGI had a 4.8 times higher value of ϕ_{oxi} and a much lower quantum efficiency of fluorescence (Φ_{fl} = 0.037) than that of fluorescein (Φ_{fl} = 0.85), suggesting that the intersystem crossing efficiency from the lowest singlet excited state to the triplet state had been greatly enhanced by the internal heavy atom effect. Cellular phototoxicity of a photosensitizer upon light illumination depends on both the extinction coefficient (ϵ) and ϕ_{oxi}, so the cellular phototoxicity of TGI-βGal ($\epsilon \cdot \phi_{oxi}$) is expected to change dramatically (about 20 times) in the presence of β-galactosidase.

We then examined whether TGI-βGal could selectively ablate β-galactosidase-expressing cells upon light illumination. HEK293 cells transduced or not transduced with *lacZ* (*lacZ*-positive or *lacZ*-negative cells) were loaded with 25 μM TGI-βGal for 2 h. Bright fluorescence was observed only in *lacZ*(+) cells (Fig. 3a–d), owing to the higher quantum efficiency of fluorescence of TGI than that of TGI-βGal (Table 1 and Supplementary Fig. 2), thereby demonstrating that TGI-βGal permeates through the plasma membrane and is converted into TGI in *lacZ*(+) cells. After wash-

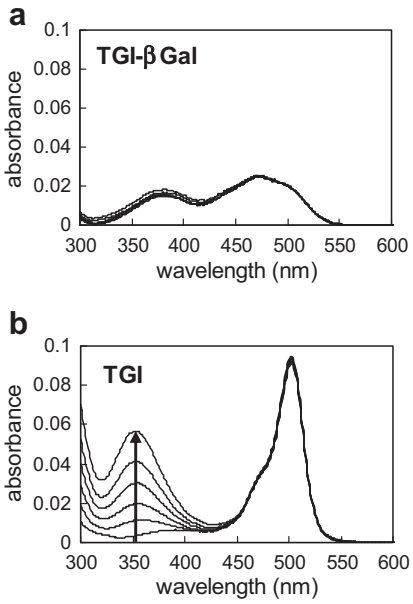


Figure 2. Time profile of absorption spectra during iodine ion oxidation. A 1 μM solution of (a) TGI-βGal or (b) TGI in 100 mM sodium phosphate buffer (pH 7.4), containing 100 mM KI was illuminated with Xe light source, which was filtered to around 490 nm, and absorption spectra were measured every 2 min.

out of excess substrate, the cells were illuminated with blue light (BP 470–490 nm, 7 mW/cm^2 at 490 nm) for 1 min. The combination of TGI-βGal, expression of *lacZ*, and light illumination resulted in cellular toxicity (Fig. 3 and Supplementary Fig. 3). Significant morphological changes were observed only in *lacZ*(+) cells (Fig. 3e and f), and the occurrence of lethal damage was confirmed by cell viability staining (Supplementary Fig. 3). These results demonstrate that TGI-βGal could selectively ablate *lacZ*-positive cells upon light illumination.

In conclusion, we have developed a β-galactosidase-activated photosensitizer, which induces oxidative stress only after having been hydrolyzed by β-galactosidase, based on quenching of oxidative stress induction by the photosensitizer via intramolecular electron transfer, and activation by enzymatic removal of the quenching substituent. The enzymatic reaction results in a 20-fold increase of oxidative stress induction ability, and selective ablation of *lacZ*-positive HEK 293 cells was demonstrated in the presence of TGI-βGal under light illumination. Activatable photosensitizers such as TGI-βGal are expected to offer unique advantages in biological and clinical applications. First, selective ablation of a cell population of interest in a temporally well controlled manner would enable us to investigate the roles of specific cell populations in neural circuits or the fate of cells in developing and adult organisms, although bystander effects[11] may limit a spatial resolution of cell ablation. Second, activatable photosensitizers would open up new possibilities for PDT without causing prolonged light sensitivity, which is a serious clinical side effect of photosensitizers that do not specifically localize to tumor tissues by designing photosensitizers which recognize environments of specific tumor. We now plan biological studies to confirm the usefulness of TGI-βGal in silencing specific populations of cells, especially neurons.

Table 1
Summary of photochemical and photosensitization properties of TGI-βGal and TGI

	Absorption max (nm)[a]	Extinction coefficient ($\times 10^4$ M^{-1} cm^{-1})[a]	Emission max (nm)[a]	Φ_{fl}[a]	Slope[b] [$\times 10^{-4}$/min] = $\varepsilon \cdot \phi_{oxi}$	ε_{490}[c] [$\times 10^4$ M^{-1} cm^{-1}]	ϕ_{oxi}[d] [$\times 10^{-8}$]	$\varepsilon \cdot \phi_{oxi}$[e] (rel.)
TGI-βGal	470	2.6	—	~0	2.6	2.2	1.2	1
TGI	502	9.4	526	0.037	52	6.4	8.2	20
Fluorescein					14	7.9	1.7	

[a] All data were measured in 0.1 M sodium phosphate buffer, pH 7.4. Quantum efficiency of fluorescence (Φ_{fl}) was determined using that of fluorescein (0.85) in 0.1 M NaOH aq as a standard.
[b] Rate of I_3^- production monitored by measuring the absorbance 351 nm in 100 mM sodium phosphate buffer, pH 7.4, containing 100 mM KI.
[c] Molar extinction coefficient at 490 nm.
[d] Relative efficiency of oxidative stress induction (ϕ_{oxi}) determined by calibrating the slope in terms of ε_{490}.
[e] The relative value of $\varepsilon \cdot \phi_{oxi}$ of TGI taking that of TGI-βGal as 1.

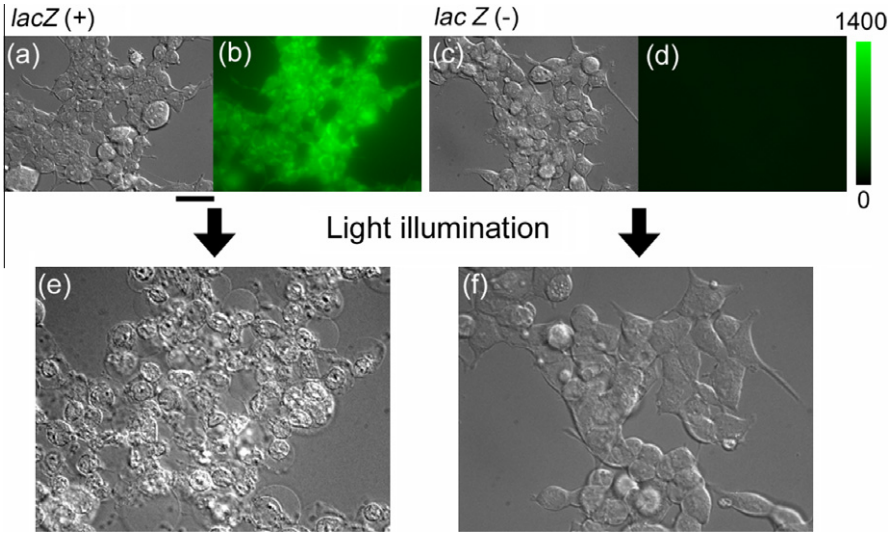


Figure 3. Selective killing of *lacZ*-positive cells using TGI-βGal. (a–d) Differential interference contrast (DIC) and fluorescence images of *lacZ*-positive HEK 293 cells (a and b) or *lacZ*-negative cells (c and d), loaded with TGI-βGal. (e) DIC images of HEK 293 cells (*lacZ*(+)) after light illumination in the presence of TGI-βGal. (f) HEK 293 cells (*lacZ*(−)) were not affected by light illumination in the presence of TGI-βGal. Scale bar indicates 5 μm.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.091.

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