Macrotheranostic Probe with Disease-Activated Near-Infrared Fluorescence, Photoacoustic, and Photothermal Signals for Imaging-Guided Therapy

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Abstract: Theranostics provides opportunities for precision cancer therapy. However, theranostic probes that simultaneously turn on their diagnostic signal and pharmacological action only in response to a targeted biomarker have been less exploited. We herein report the synthesis of a macrotheranostic probe that specifically activates its near-infrared fluorescence (NIRF), photoacoustic (PA), and photothermal signals in the presence of a cancer-overexpressed enzyme for imaging-guided cancer therapy. Superior to the small-molecule counterpart probe, the macrotheranostic probe has ideal biodistribution and renal clearance, permitting passive targeting of tumors, in situ activation of multimodal signals, and effective photothermal ablation.

Theranostics, which integrates diagnostic and therapeutic capabilities into a single entity, possesses the potential to detect disease at early stage, assess the accumulation of therapeutic agents at disease site, and predict therapeutic outcomes.[1] Thereby, theranostics provides opportunities for precision medicine.[2] However, because most existing systems simply combine therapeutic agents with imaging probes through nano-encapsulation or bioconjugation, their diagnostic signals and pharmacological effect are always on.[3] Thus, the efficacies of such theranostic probes strongly rely on the difference in the concentration of the probe between disease tissue and normal tissue, which inevitably results in signal interference and causes side effects in normal tissues.[4]

Ideal theranostic probes should undergo intrinsic signal evolution accompanied by simultaneous initiation of pharmacological action only upon detection of a targeted biomarker in living systems.[5] However, such activatable theranostic probes are rare. Until now, enzyme-, [6a] pH-, [6b] glutathione (GSH)-, [6c-d] and reactive oxygen species (ROS)-responsive[6e] activatable theranostic probes have been developed for cancer therapy, but those probes are limited to the simple design of combining activatable fluorescent probes with chemotherapy prodrugs.[6] In addition, activatable theranostic probes with photothermal therapy (PTT) capability have been less exploited, although PTT has the advantage of high spatial-temporal controllability and minimal invasiveness.[7]

Herein, we report the design and synthesis of an activatable macrotheranostic probe that specifically turns on its near-infrared fluorescence (NIRF), photoacoustic (PA), and photothermal signals in the presence of cancer for imaging-guided therapy. The macrotheranostic probe (CyGal-P) is composed of a d-galactose-caged NIR hemicyanine dye (CyOH) linked with a long poly(ethylene glycol) (PEG) chain (Scheme 1a). The d-galactose-caged moiety can be specifically cleaved at the glycosidic bond by β-galactosidase.
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the photothermal properties of CyGal-P and CyGal in the absence or presence of βGal had a similar tendency (Figure 1d). Under continuous laser irradiation at 680 nm, βGal-treated CyGal-P and CyGal showed gradually increased temperatures and reached plateau at \( t = 180 \) s; in contrast, the temperatures without βGal treatment increase little owing to the low absorption of the inactivated probes at 680 nm. The maximum photothermal temperatures of βGal-activated CyGal-P and CyGal were 48 and 37°C, respectively (Figure S12a, Supporting Information). The reversible heating–cooling operation showed that βGal-activated CyGal-P remained nearly the same for at least 5 cycles, while βGal-activated CyGal failed to do so (Figure S12b, Supporting Information). The absorption spectra before and after light irradiation revealed that the absorption at 688 nm dramatically decreased for CyGal but remained nearly the same for CyGal-P (Figure S12c,d, Supporting Information). This indicated that the presence of PEG also enhanced the photothermal stability of uncaged NIR dye, probably owing to the faster heat dissipation of well-dissolved dye relative to the aggregated dye. These data confirmed that the macrotheranostic probe activated its PA and photothermal signals in the presence of βGal.

To test the response of the macrotheranostic probe toward βGal in cells, fluorescence imaging was conducted on the βGal-overexpressed ovarian cancer cells (SKOV3) and the control cells (NIH-3T3). Strong NIR fluorescence signals for both CyGal-P- and CyGal-treated SKOV3 cells were detected after 1 h incubation (Figure S13a, Supporting Information). However, the fluorescence for CyGal-P-treated SKOV3 cells was 2.1-fold of that for CyGal-treated SKOV3 cells (Figure S13b, Supporting Information), consistent with the solution results. In contrast, the fluorescence signals were barely observed for NIH-3T3 cells after probe treatment (Figure S13a, Supporting Information). These results confirmed the macrotheranostic probe was specifically activated by βGal in cells.

To examine the PTT efficacy of the macrotheranostic probe in vitro, SKOV3 cells were incubated with CyGal-P or CyGal for 1 h and then irradiated for 5 min with a 680 nm laser (0.6 W cm\(^{-2}\)). The cell status was qualitatively evaluated by calcein AM (live cells, green fluorescence) and propidium iodide (dead cells, red fluorescence) staining (Figure S14, Supporting Information), as well as quantitatively measured by using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assays (Figure S15, Supporting Information). Both CyGal-P and CyGal had no obvious cytotoxicity without laser irradiation. Owing to the efficient cleavage of CyGal-P and better photothermal stability after βGal activation, high temperatures (48°C) were achieved to ablate almost all the SKOV3 cells under laser irradiation (Figure 1d, Figure S14 and Figure S15, Supporting Information). In contrast, CyGal failed to do so.

To evaluate the in vivo biodistribution of the probes, CyGal-P and CyGal were injected into living mice by intravenous injection (Figure S16, Supporting Information). Despite the weak fluorescence (Figure 1b), the NIRF signals could still be detected for the biodistribution study. CyGal-P had higher accumulation in kidneys and could be cleared out through renal excretion, while CyGal was mainly accumulated in the liver. These data revealed that the enhanced water-solubility of the macrotheranostic probe (CyGal-P) helped the escape from mononuclear phagocytic system (MPS) and facilitated renal clearance.\(^{[11]}\)

The in vivo tumor NIRF and PA imaging capabilities of the macrotheranostic probe (CyGal-P) were tested in the subcutaneous SKOV3 xenograft tumor model. After systemic administration of CyGal-P or CyGal into the living mice through tail vein, both NIRF and PA images were longitudinally recorded and quantified (Figures 2a–d). The NIRF and PA intensities gradually increased for CyGal-P over time and reached maximum values at 60 min post-injection, indicating that CyGal-P could efficiently accumulate in the tumor region and undergo cleavage by βGal overexpressed in SKOV3 cells. In contrast, owing to its poor water-solubility, CyGal showed very low accumulation in the tumor region, failing to delineate the tumor. The in vivo PA spectrum of the tumor from the CyGal-P treated mice resembled the solution spectrum of βGal-activated CyGal-P (Figure 1c and Figure 2e), while the spectrum of the tumor from the CyGal-treated mice was similar to the background. These data verified that the increased PA signals in the tumor region came from βGal-activated CyGal-P.

Because CyGal-P had the highest activated NIRF and PA signals in the tumor region at 60 min post-injection, PTT was...
conducted at this time point. The SKOV3-tumor-bearing mice were irradiated at 680 nm (0.6 W cm\(^{-2}\)) for 5 min. The tumor temperature for CyGal-P-treated mice gradually increased and reached a plateau at 4 min, which was significantly higher than that for both CyGal and saline-treated mice at all the time points (Figure 3a,b). The tumor temperature for CyGal-treated mice reached maximum at 2 min and then dropped owing to the decomposition of CyGal during the laser irradiation (Figure S12c, Supporting Information). The maximal tumor temperature of CyGal-P-treated mice was 48.3°C, which was 9.5 and 9.3°C higher than that of CyGal- and saline-treated mice. These data indicated that the amount of βGal-activated CyGal-P in tumor was high enough to induce the photothermal heating above the threshold temperature (43°C) for cellular ablation, which was impossible for CyGal.[12]

To qualitatively investigate the PTT efficacy of the macrotheranostic probe, the growth rates of tumors were continuously monitored after PTT (Figure 3c). Owing to the higher accumulation in tumor and higher photothermal temperature for CyGal-P, CyGal-P successfully suppressed the tumor growth after PTT, while CyGal failed to do so (Figure S17, Supporting Information). No therapeutic effect was observed for the groups without laser irradiation. Moreover, for mice in all groups, no significant weight loss was observed for 25 days after PTT (Figure 3d), and no noticeable histopathological abnormalities were found in livers, kidneys, and spleens (Figure S18, Supporting Information), showing the good biosafety of both probes. Immunofluorescence caspase-3 staining images revealed that a large fraction of green fluorescence spots were observed for the tumor tissues of CyGal-P-treated mice after PTT (Figure 3e), while few green fluorescence spots were found for other control groups (Figure S19, Supporting Information), indicating that only CyGal-P-mediated PTT led to the severe cellular apoptosis.[13]

Therefore, these data verified that the macrotheranostic probe had a specific PTT efficacy and minimal toxicity to normal tissues.

In conclusion, we synthesized a macrotheranostic probe (CyGal-P) that could be specifically activated by an ovarian-cancer-overexpressed enzyme (βGal) to turn on its NIRF, PA, and photothermal signals for imaging-guided cancer therapy. CyGal-P is different from most reported theranostics agents whose signal and pharmacological action are always on.[3] Moreover, as compared with the small-molecule counterpart probe (CyGal), CyGal-P showed significantly higher fluorescence (47-fold vs. 24-fold), PA (564 vs. 370), and photothermal (48°C vs. 37°C) enhancement after activation by βGal. With the help of hydrophilic PEG, CyGal-P also had the ideal biodistribution with renal clearance and passive accumulation in tumor, which was not possible for CyGal. As a result, only CyGal-P could delineate the tumor with both NIRF and PA imaging and activate its photothermal heat to ablate the βGal-overexpressed ovarian cancer cells (SKOV3) in living mice after systemic administration. To the best of our knowledge, our study represents the first example of an activatable phototheranostic agent with multimodal imaging and PTT capabilities.

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**Conflict of interest**

The authors declare no conflict of interest.

**Keywords**: activatable macrotheranostic probes · near-infrared fluorescence imaging · photoacoustic imaging · photothermal therapy · β-galactosidase


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An activatable macrotheranostic probe was designed for imaging-guided photothermal therapy. The probe could specifically turn on near-infrared fluorescence (NIRF), photoacoustic (PA), and photothermal signals in the presence of a cancer-overexpressed enzyme (β-galactosidase) for imaging-guided cancer therapy.
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