Antitumor Effects in Gastrointestinal Stromal Tumors Using Photodynamic Therapy with a Novel Glucose-Conjugated Chlorin<br>

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Abstract

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract. Except for surgical resection, no effective treatment strategies have been established. Photodynamic therapy (PDT) consists of intravenous administration of a photosensitizer, activated by a specific wavelength of light, which produces reactive oxygen species that directly kill tumor cells. We analyzed the efficacy of PDT using a newly developed photosensitizer, 5,10,15,20-tetrakis-[4-[β-D-glucopyranosylthio]-2,3,5,6-tetrafluorophenyl]-2,3,6-[methanophenyl]-2,3,5,6-tetrafluorophenyl]-2,3,6-methano[iminomethano] chlorin (H2TFPC-SGlc), for the GIST treatment. Various photosensitizers were administered in vitro to GIST (GIST-T1) and fibroblast (WI-38) cells, followed by irradiation, after which cell death was compared. We additionally established xenograft mouse models with GIST-T1 tumors and examined the accumulation and antitumor effects of these photosensitizers in vivo. In vitro, the expression of the glucose transporters GLUT1, GLUT3, and GLUT4, the cellular uptake of H2TFPC-SGlc, and apoptosis mediated by PDT with H2TFPC-SGlc were significantly higher in GIST-T1 than in WI-38 cells. In vivo, H2TFPC-SGlc accumulation was higher in xenograft tumors of GIST-T1 cells than in the adjacent normal tissue, and tumor growth was significantly suppressed following PDT. PDT with novel H2TFPC-SGlc is potentially useful for clinical applications about the treatment of GIST. Mol Cancer Ther; 13(4); 1–9. © 2014 AACR.

Introduction

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the digestive tract. GIST cells are believed to originate from either progenitors of the interstitial cells of Cajal (1, 2), a population of spindle-shaped cells that are the pacemaker cells of the gut, or from an interstitial mesenchymal precursor stem cell (3, 4). GISTs >2 cm in diameter are typically resected, whereas GISTs <2 cm in diameter are monitored closely for rapid growth or metastasis (5–7). As no effective treatments other than surgical resection are available, it is necessary to elucidate new therapies and approaches for the treatment of GISTs, particularly when they are small (8).

One such approach, photodynamic therapy (PDT), has several advantages over conventional cancer treatments. PDT consists of the intravenous administration of a photosensitizer, which preferentially localizes within the tumor, followed by activation with a specific wavelength of light (9). Activation of the photosensitizer causes the conversion of molecular oxygen into various reactive oxygen species (ROS) that directly induce the death of the tumor cells or damage the tumor-associated vasculature (10). PDT is relatively noninvasive and has a lower systemic toxicity because irradiation and activation occur only at the tumor site (10, 11). Thus, PDT has been widely used to treat various tumors, which can be directly reached by different wavelengths of light, such as lung, esophageal, gastric, breast, head and neck, bladder, and prostate cancer (9). Compared with other therapies, PDT often produces a high cure rate with a low recurrence rate (12). Photofrin, a first-generation photosensitizer, is widely used in the clinic (10, 11); however, talaporfin, a second-generation photosensitizer, has several advantages over photofrin, including decreased prolonged photosensitization (13). Talaporfin-mediated PDT has been examined in the treatment of several solid tumors (14, 15). However, the insufficiency of efficacy and skin photosensitivity...
remains unsolved, so the more effective photosensitizer is expected to be developed.

In this study, we evaluated the efficacy of PDT with a new glucose-conjugated photosensitizer, glycoconjugated chlorin(5,10,15,20-tetrakis-[4-β-D-glucopyranosylthio-2,3,5,6-tetrafluorophenyl]-2,3-[methano[N-methyl]iminomethano]chlorin, H2TFPC-SGlc) for the treatment of GIST in vitro and in vivo. As GIST cells readily take up glucose in positron emission tomography scans, and the long wavelengths of the light spectrum (red, 630–670 nm) can penetrate to the deep layers of the stomach wall, PDT consisting of the administration of H2TFPC-SGlc with activation at 660 nm is a good candidate for GIST treatment.

In our previous study, we indicated that H2TFPC-SGlc was able to induce apoptosis via singlet oxygen, was approximately 30 times more cytotoxic than talaporfin during PDT, and could be a potential photosensitizer of PDT of gastric and colon cancer in vitro and in vivo as we believe that it has superior cancer cell selectivity and specificity (16).

Therefore, in this study, we evaluated the efficacy of PDT with a new photosensitizer—glucose-conjugated chlorin (H2TFPC-SGlc)—for the treatment of GIST in vitro and in vivo.

Materials and Methods

Photosensitizers

5,10,15,20-tetrakis(pentafluorophenyl)-2,3-(methano[N-methyl]iminomethano)chlorin (H2TFPC) and H2TFPC-SGlc (Fig. 1A) were synthesized and provided by the laboratory of the Kyoto University (Japan) and Okayama University of Science (Japan). They contain no isomers, based on 1H-NMR and 19F-NMR measurements (Supplementary Data S1).

Cell culture

The GIST-T1 cell line has been characterized in detail and was provided on July 14, 2011 by Taguchi and colleagues (17). WI-38 cells (Japanese Cancer Research Resources Bank, No. IFO50075), which are human embryonic fibroblasts derived from the lung, were cultured in Eagle minimum essential medium (Wako. Pure Chemical Industries Co. Ltd.) supplemented with 10% FBS and 1% ampicillin and streptomycin under 5% CO2 at 37°C. The human GIST cell line, GIST-T1, was cultured in normal (1,000 mg/L) or high (4,500 mg/L) glucose Dulbecco’s Modified Eagle Medium (Wako Pure Chemical Industries Co. Ltd.) supplemented with 10% FBS and 1% ampicillin and streptomycin under 5% CO2 at 37°C (Fig. 1B).

Western blotting

Cells were washed with PBS (Sigma) 3 times and dissolved in 1 mL of cell lysis buffer (Cell Signaling Technology) containing 20 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1 mmol/L Na2EDTA, 1 mmol/L EGTA, 1% Triton, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L Na3VO4, and 1 μg/mL leupeptin. One millimolar phenylmethylsulfonyl fluoride was added directly before use. Cells were disrupted for

Figure 1. Chemical structure of photosensitizers and characteristics of cell lines. A, the structures of 5,10,15,20-tetrakis(pentafluorophenyl)-2,3-(methano[N-methyl]iminomethano)chlorin (H2TFPC, left) and H2TFPC-SGlc (Fig. 1A) are shown. B, the morphologies of GIST-T1 and WI-38 cells were determined by microscopy. (magnification, ×200). C, c-Kit expression of GIST-T1 and WI-38 cells was investigated at the protein level by Western blotting. β-Actin was used as a loading control.
15 seconds on ice using a Bio-ruptor sonicator (Cosmo Bio) and centrifuged at 15,000 rpm for 10 minutes at 4°C. Each sample was normalized to an equal protein concentration using a Protein Assay Kit (Bio-Rad Laboratories). An equal quantity of 2× SDS-PAGE sample buffer [0.5 M Tris-HCl (pH 7.2), 1% SDS, 100 mmol/L β-mercaptoethanol, and 0.01% bromophenol blue] was added to each sample, and samples were boiled for 5 minutes at 100°C. Aliquots of each sample were separated by SDS-PAGE on an 8% to 15% gel and transferred on to a nitrocellulose membrane. The membrane was blocked with 5% skim milk in PBS for 1 hour at room temperature, followed by incubation with the primary antibodies anti-GLUT1, anti-GLUT3, or anti-GLUT4 (Santa Cruz Biotechnology, Inc., Incubation with the primary antibodies anti-GLUT1, anti-GLUT3, or anti-GLUT4 (Santa Cruz Biotechnology, Inc., 1:2,000) overnight at 4°C. The membrane was washed with 0.05% Tween 20 in PBS 3 times at 5-minute intervals, incubated with secondary antibody for 1 hour at room temperature, and washed again with 0.05% Tween 20 in PBS 3 times at 5-minute intervals. The membrane was incubated with enhanced chemiluminescence detection reagents (Amersham) for 30 minutes at 37°C. After that, the membrane was washed with 0.05% Tween 20 in PBS 3 times at 5-minute intervals, exposed to scientific imaging film (Eastman Kodak), and then developed with an X-ray film (Kodak). The bands were visualized and analyzed using the ImageJ program (National Institutes of Health, Bethesda, MD).

**Intracellular localization**

Cells were incubated with H2TFPC (1 μmol/L, 20 μmol/L) or H2TFPC-SGlc (1 μmol/L) at 4 hours and stained with organelle-specific fluorescent probes. Lysosomes were labeled with 0.1 μmol/L LysoTracker Green (Invitrogen) for 30 minutes at 37°C. Mitochondria were labeled with 0.1 μmol/L MitoTracker Green FM (Invitrogen) at 37°C for 10 minutes. Golgi bodies were stained with 5 μmol/L NBD C6-ceramide at 4°C for 30 minutes. The endoplasmic reticulum was labeled with 0.1 μmol/L ER-Tracker Green (Invitrogen) at 37°C for 30 minutes. Stained cells were observed with a confocal laser microscope (Nikon A1 confocal system; Nikon Instech Co., Ltd.), and data were analyzed using NIS element imaging software (Nikon). Bandpass emission filters of 505 to 530 nm and 650 nm were used.

**In vitro PDT**

GIST-T1 and WI-38 cells were incubated with H2TFPC or H2TFPC-SGlc in cell culture medium for 4 hours. Cells were washed once with PBS, covered with PBS, and irradiated with of LED light (OptoCode Corporation) at 660 nm. Following irradiation, the PBS in the wells was exchanged for medium supplemented with 2% FBS, and the cells were incubated for the specified times before analyses. The results between cells were analyzed by the Welch t test.

**Cell viability assay**

Cell viability was analyzed by the WST-8 cell-proliferation assay. GIST-T1 and WI38 cells were seeded into 96-well culture plates at a concentration of 5 × 10³ cells/100 μL/well and incubated overnight. Cells were incubated with H2TFPC or H2TFPC-SGlc at 37°C for 4 hours, irradiated, and further incubated for 24 hours. To determine survival, cells were incubated with the Cell Counting Kit-8 (Dojindo) according to manufacturer’s protocol for 4 hours, and the absorbance at 450 nm was measured with a microplate spectrophotometer (SPECTRA MAX340; Molecular Devices). Cell viability was expressed as a percentage of untreated control cells. The results between cells were analyzed by the Welch t test.

**Animals and tumor models**

Pathogen-free female nude mice (BALB/c Slc-nu/nu), 6 to 8 weeks of age, with a body weight of 20 to 25 g, were obtained from Japan SLC. Xenograft tumor models were established by subcutaneously implanting 5 × 10⁶ cells.
GIST-T1 cells in 200 µL of PBS. The procedures in these experiments were approved by Nagoya City University Center for Experimental Animal Science, and mice were cared for according to the guidelines of the Nagoya City University for Animal Experiments.

**Spectrophotometric analysis device**

We examined the accumulation of H2TFPC and H2 TFPC-SGlc in the xenograft tumor model using a semiconductor laser with a VLD-M1 spectrometer (M&M Co., Ltd.) that exposed a laser light with a peak wavelength of 405 ± 1 nm and a light output of 140 mW. The spectrometer and its accessory software (BW-Spec V3.24; B&W TEK, Inc.) were used to analyze the spectrum waveform, which revealed an amplitude peak (relative fluorescence intensity) of 505 nm for autofluorescence and 655 nm for H2TFPC-SGlc. To reduce measurement error, we compared the relative fluorescence intensity ratios of H2TFPC-SGlc in the target tissues, which were calculated by dividing the relative fluorescence intensity by that of autofluorescence. The results from the tumor and adjacent normal tissue were compared by the Welch t test.

**In vivo PDT**

Mice were administered H2TFPC or H2TFPC-SGlc by tail vein injection at a dose of 1.25 µmol/kg. Four hours after injection, the tumors were irradiated with 660 nm LED light (OptoCode Corporation) at a dose of 40 J/cm² (intensity: 49 mW/cm²) to the skin directly above the tumor. Treatment was repeated 4 times at 10, 17, 24, and 31 days after tumor inoculation. Tumor growth was monitored daily by measuring the tumor volume with vernier calipers, and the tumor volume was calculated by the formula length × width × depth/2. The results were analyzed by the Bonferroni–Holm method to assess differences between groups.

**Statistical analysis**

Descriptive statistics and simple analyses were performed using the statistical package R, version 2.4.1 (www.r-project.org/). In all analyses, P-values < 0.05 were considered statistically significant.

**Results**

**A GIST cell line, GIST-T1, expresses c-Kit**

The GIST-T1 cell line was strongly positive for c-Kit, but the fibroblast cell line WI-38 was not, as shown in Fig. 1C. The expression of c-Kit has emerged as the most important defining feature of GIST and probably the gold standard for diagnosing GIST (19).

**Expression of glucose transporters in cell lines**

Expression of glucose transporters in these cells depends on the extracellular glucose concentration. We analyzed the expression of GLUT1, GLUT3, and GLUT4 protein and mRNA in GIST-T1 and WI-38 cells using Western blot analysis and RT-PCR analysis. GLUT1, GLUT3, and GLUT4 protein and mRNA expression increased significantly in GIST-T1 and WI-38 cells when cultured in normal glucose medium compared with high glucose medium. GIST-T1 cells significantly expressed higher GLUT1, GLUT3, and GLUT4 protein and mRNA than WI-38 cells in both normal and high glucose culture conditions (Fig. 2).

**Cellular uptake of 2-NBDG, H2TFPC, and H2TFPC-SGlc**

We first examined the uptake of 2-NBDG, H2TFPC, and H2TFPC-SGlc using GIST-T1 and WI-38 cells. Cells were incubated with 1 µmol/L 2-NBDG, H2TFPC, or H2 TFPC-SGlc in normal or high glucose medium for the indicated times, and uptake was estimated by measuring the intensity of the characteristic red fluorescence at the single cell level using FACS. As shown in Fig. 3A (normal glucose medium) and 3B (high glucose medium), the uptake of 2-NBDG and H2TFPC-SGlc was higher in GIST-T1 than in WI-38 cells. There was no apparent difference in the uptake of H2TFPC between GIST-T1 and WI-38 cells. These results indicated that glucose conjugation to chlorin induced greater GIST cell specificity and selectivity. We tried to culture GIST-T1 cells in non-glucose medium, but GIST-T1 cells did not grow for a week in non-glucose medium. GIST-T1 cells in non-glucose medium for 4 hours did not change the uptake of H2TFPC-SGlc from in normal glucose medium (Supplementary Fig. S1).

**Subcellular localization of H2TFPC-SGlc**

Next, we investigated the subcellular localization of H2 TFPC-SGlc using fluorescence probes for intracellular organelles. Cells were loaded with H2TFPC-SGlc and incubated with MitoTracker Green, LysoTracker Green, NBD C6-ceramide Green, or ER-Tracker Green to label the mitochondria, lysosomes, Golgi bodies, or endoplasmic reticulum, respectively. H2TFPC-SGlc colocalized with MitoTracker and ER-Tracker, indicating that the photosensitizer accumulated in the mitochondria and endoplasmic reticulum (Fig. 3C). In addition, we also examined the subcellular localization of H2TFPC. The expression level was low at 1 µmol/L, but H2TFPC (20 µmol/L) was also mainly accumulated in the mitochondria and endoplasmic reticulum (Supplementary Fig. S2). These things suggested that chlorin (H2TFPC) tends to be localized in the mitochondria and endoplasmic reticulum regardless of the conjugation of glucose.

**PDT with H2TFPC-SGlc induced cell death through apoptosis**

We examined PDT-induced cell death with H2TFPC and H2TFPC-SGlc in normal (Fig. 4A) and high glucose medium (Fig. 4B). GIST-T1 and WI-38 cells were incubated with H2TFPC and H2TFPC-SGlc for 4 hours and irradiated with 16 J/cm² of 660 nm LED light. PDT with H2 TFPC-SGlc displayed significantly stronger toxicity than PDT with H2TFPC and induced cell death more efficiently in GIST-T1 compared with WI-38 cells. The cytotoxicity of PDT with H2TFPC-SGlc in GIST-T1 cells increased with increasing light doses in normal (Fig. 4C) and high glucose medium (Fig. 4D). There was no apparent difference in
toxicity between the normal and high glucose medium. We also measured the mean fluorescence intensity of active caspase-3 by FACS as a marker for apoptosis. Mean fluorescence intensity increased at 4 hours after irradiation and peaked at 16 hours. H$_2$TFPC-SGlc–mediated PDT induced apoptosis more efficiently in GIST-T1 than in WI-38 cells. There was no detectable difference in apoptosis induction between normal (Fig. 4E) and high glucose media (Fig. 4F).

**Accumulation of H$_2$TFPC-SGlc in GIST tumors**

We examined the ability of H$_2$TFPC-SGlc to accumulate in xenograft GIST tumors established by subcutaneously implanting GIST-1 cells. Following tail vein injection of either 1.25 μmol/kg H$_2$TFPC or H$_2$TFPC-SGlc, the spectrum waveform from the xenograft tumors was analyzed by a VLD-M1 spectrophotometer. The spectrum waveform showed 2 peaks of fluorescence emission spectra, one at 505 nm, corresponding to autofluorescence, and one at 655 nm, corresponding to either H$_2$TFPC or H$_2$TFPC-SGlc. We then measured the relative fluorescence intensity ratio of H$_2$TFPC or H$_2$TFPC-SGlc in the tumors and the adjacent normal tissue. The relative fluorescence intensity ratio of H$_2$TFPC-SGlc was highest 4 hours after drug administration. H$_2$TFPC-SGlc accumulated in the tumor tissue at a significantly higher rate than in the adjacent normal tissue, but H$_2$TFPC did not (Fig. 5A). Furthermore, we measured the biodistribution of H$_2$TFPC and H$_2$TFPC-SGlc in xenograft models by using spectrophotometer. Significant accumulation of H$_2$TFPC-SGlc to the tumor tissue was detected. Among the organ tissues, H$_2$TFPC-SGlc tends to accumulate in liver and kidney, but these accumulations were much lower than that in tumor (Supplementary Data S2).

**Antitumor effects of H$_2$TFPC-SGlc in vivo**

To determine the antitumor effects of H$_2$TFPC-SGlc on xenograft tumors in mice, mice were administered H$_2$TFPC or H$_2$TFPC-SGlc at a dose of 1.25 μmol/kg by i.v. 10 days after tumor inoculation and then irradiated with 660 nm LED light at 40 J/cm$^2$. The tumor sizes before irradiation were 20 to 50 mm$^3$. H$_2$TFPC-SGlc–mediated PDT was repeated every 7 days for 4 cycles. Analysis determined that there was damage to the tumor without damage to the adjacent normal tissues. H$_2$TFPC-SGlc–mediated PDT ($n = 5$) suppressed tumor growth significantly...
compared with the control treatment (light alone) and H₂TFPC-mediated PDT (P < 0.01; Fig. 5B). The treatments had no obvious side effects, such as diarrhea and/or weight loss (data not shown). Single H₂TFPC-SGlc–mediated PDT also significantly suppressed tumor growth (P < 0.01; Supplementary Fig. S3).

Figure 3. Uptake and subcellular localization of H₂TFPC-SGlc. A and B, GIST-T1 and WI-38 cells were incubated in normal (A) or high (B) glucose medium with 1 µmol/L of glucose (2-NBDG), H₂TFPC, or H₂TFPC-SGlc for various timepoints, and the uptake of the drugs was estimated by FACS. Data are the mean fluorescence intensity ± SE. **, P < 0.01; *, P < 0.05. C, GIST-T1 cells were loaded with H₂TFPC-SGlc (1 µmol/L) for 4 hours and labeled with MitoTracker Green, LysoTracker Green, NBD-C6 ceramide Green or ER-Tracker Green. The images were obtained by confocal microscopy (original magnification ×1,000; scale bar, 10 µm).

Figure 4. Cell death by PDT. A–D, GIST-T1 and WI-38 cells were incubated in normal (A) or high (B) glucose medium with various concentrations of H₂TFPC or H₂TFPC-SGlc, irradiated with 16 J/cm² of 660 nm LED light, and incubated for 24 hours. GIST-T1 cells in normal (C) or high (D) glucose medium were incubated with 0.1 or 0.2 µmol/L of H₂TFPC-SGlc and irradiated with various doses of 660 nm LED light. Cell viability was determined by a WST-8 assay. Data are means of 3 independent experiments ± SE. **, P < 0.01; *, P < 0.05. E and F, GIST-T1 or WI-38 cells in normal (E) or high (F) glucose medium were incubated with 1 µmol/L of H₂TFPC-SGlc, irradiated with 16 J/cm² of 660 nm LED light, and incubated for various lengths of time. Cells were stained with FITC-labeled active caspase-3 antibody, and apoptosis was analyzed through FACS. Data are means of 3 independent experiments ± SE. **, P < 0.01; *, P < 0.05.
H2TFPC-SGlc, a chlorin-based photosensitizer, was expected to have a number of advantages in PDT, including significant reductions in dark cytotoxicity, improved water-solubility, greater cellular uptake, and sugar-dependent photocytoxicity over currently used photosensitizers (20–22). In a previous study, we have reported that H2TFPC was 30 times more cytotoxic to gastric cancer cells in vitro as compared with the second-generation photosensitizer, talaporfin. Moreover, in xenograft tumors, H2TFPC-SGlc accumulation was higher and significantly suppressed tumor growth as compared with talaporfin (16).

In this study, we investigated whether H2TFPC-SGlc could act as a potential photosensitizer of PDT in GIST in vitro and in vivo. In vitro, H2TFPC-SGlc-mediated PDT was shown to induce cell death via apoptosis. In vivo, H2TFPC-SGlc-mediated PDT suppressed tumor growth and produced no observable adverse effects on normal adjacent tissues. These results indicate that PDT with H2TFPC-SGlc is a minimally invasive therapeutic modality for clinical treatment of GIST. We used the GIST-T1 cell line, which was established from a patient with metastatic GIST by Taguchi and colleagues (17). GIST-T1 cell line expresses c-Kit oncogene and is considered to be one of the most representative GIST cell lines that mimic the nature of human GIST. In addition, GIST-T1 is the only cell line that can be used for a xenograft model in vivo at this point (Fig. 1C).

There have been many attempts to develop new photosensitizers that show preferential accumulation within the target tumor tissue through conjugation with various active targeting approaches, such as conjugation with peptides or antibodies (23–25), incorporation within liposomes (26, 27), and encapsulation within polymeric nanoparticles (28–31). To initiate accumulation in the target tumor, H2TFPC-SGlc was developed by linking glucose to the photosensitizer chlorin.

In vitro, the uptake of H2TFPC-SGlc in GIST-T1 cells was much greater than that in normal WI-38 cells. The uptake of 2-NBDG was also significantly higher in GIST-T1 cells than in WI-38 cells; however, there was no changes in H2TFPC uptake between GIST-T1 and WI-38 cells (Fig. 3A and B).

In vivo, H2TFPC-SGlc significantly accumulated to a greater extent in the tumor tissue than in the adjacent normal tissue (Fig. 5A). These results indicate that the linkage of glucose may be a useful tool for drug delivery in GIST-T1 as well as gastric cancer cells.

GLUT1 is believed to maintain basal glucose transport in most cell types (32–34) and seems to be the predominant glucose transporter in GIST cells. 

Figure 5. Accumulation in tumors and inhibition of tumor growth by PDT. GIST cells were inoculated in the dorsal skin of mice at a concentration of 5 × 10^6 cells/200 μL in PBS. H2TFPC or H2TFPC-SGlc at a concentration of 1.25 μmol/kg was administered by tail vein injection into tumor-bearing mice. A, the relative intensity of H2TFPC or H2TFPC-SGlc and autofluorescence were measured using a spectrometer at various timepoints after injection. The relative fluorescence intensity ratio in the tumor or adjacent normal tissue was calculated by dividing the relative fluorescence intensity of photosensitizers by that of autofluorescence. Data are means ± SE (n = 5 for H2TFPC and n = 5 for H2TFPC-SGlc). **, P < 0.01; ***, P < 0.05. B, mice were irradiated with 40 J/cm² of LED light at 660 nm 4 hours after injection. Treatment was repeated 4 times at 10, 17, 24, and 31 days after tumor inoculation. Tumor volumes were monitored for 42 days total. Data are means ± SE (n = 5 for H2TFPC and n = 5 for H2TFPC-SGlc). **, P < 0.01.
glucose transporter in many types of cancer cells, although the expressions of GLUT2, GLUT3, and GLUT4 have been detected in cancer cells by immunohistochemistry or RNA analysis (34–37). We tried to examine the role of GLUT in H$_2$TFPC-SGlc uptake by knockdown of GLUT1, 2, and 4. However, knockdown of GLUT1, 2, and/or 4 did not change the uptake of H$_2$TFPC-SGlc. We speculate that GLUT2 and/or GLUT4 substitutes under silencing GLUT1, and vice versa. GLUT1 expression often correlates with the ability to detect tumors by positron emission tomography (38–40). Rapid uptake of glucose (as evidenced by positron emission tomography) in GIST typically associated with KIT mutations and increased cell survival (41). In colon cancer, GLUT1 expression is recently speculated to be the most frequently increased transcripts with the KRAS and BRAF mutation (42). In gastric cancer, GLUT1 has been reported to be expressed during gastric carcinogenesis (43, 44) and very recently gastric cancers with microsatellite instability exhibit high fluorodeoxyglucose uptake on positron emission tomography (45). Because the expressions of GLUT1, GLUT3, and GLUT4 in GIST-T1 cells were significantly higher than those in WI-38 cells, these 3 glucose transporters may play crucial roles in the cellular uptake of H$_2$TFPC-SGlc into GIST-T1 cells (Fig. 2).

Intense research has been performed to identify the molecular functions that regulate the crosstalk between apoptosis and the other major cell death subroutines (e.g., necrosis and autophagic cell death). The function of the necrotic pathway was initiated by endoplasmic reticulum/Golgi body photodamage, the apoptotic pathway by mitochondrial photodamage, and the autophagic pathway by endoplasmic reticulum photodamage (46). We observed that PDT with H$_2$TFPC-SGlc induced apoptotic cell death in GIST cells (Fig. 4E and F). As shown in Fig. 3C, H$_2$TFPC-SGlc accumulated in mitochondria and the endoplasmic reticulum. It remains a possibility that PDT with H$_2$TFPC-SGlc induces cell death by necrosis and/or autophagy.

Previous PDT experiments with the photosensitizers photofrin and/or talaporfin used irradiation doses of $>$100 J/cm$^2$ in carcinoma xenografts models (47–49). In our study, H$_2$TFPC-SGlc-mediated PDT showed antitumor effects in vitro and in vivo with a relatively low irradiation dose of 40 J/cm$^2$. This indicated that the high cancer cell selectivity and specificity of H$_2$TFPC-SGlc could reduce the total energy of light irradiation needed in H$_2$TFPC-SGlc–mediated PDT. This reduction may further reduce the side effects related to the damage of adjacent normal tissue.

As chemotherapy and/or radiation therapy are not solely effective for GIST treatment, novel treatments for GIST have been explored. In this study, we conclude that H$_2$TFPC-SGlc–mediated PDT had specificity for GIST-T1 cells and effectively suppressed the growth of xenograft tumors through apoptosis without observable damage to the adjacent normal tissue. Based on the properties and characteristics of H$_2$TFPC-SGlc presented in this study, we suggest that H$_2$TFPC-SGlc is a potential photosensitizer for PDT of GIST.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
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Development of methodology: M. Tanaka, H. Kataoka, S. Yano, S. Hamano, E. Kubota
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Tanaka, H. Ohb, S. Hamano, E. Kubota
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