Published in final edited form as:

Cancer Sci. 2007 November ; 98(11): 1727–1733. doi:10.1111/j.1349-7006.2007.00602.x.

Targeted optical fluorescence imaging of human ovarian adenocarcinoma cells using a galactosyl serum albumin-conjugated fluorophore

Andrew J. Gunn^{a,b}, Yukihiro Hama^a, Yoshinori Koyama^a, Elise C. Kohn^c, Peter L. Choyke^a, and Hisataka Kobayashi^{a,*}

^a Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, NIH, 10 Center Drive, Bethesda, MD 20892-1088, USA

^b Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815-6789, USA

^c Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, NIH, 10 Center Drive, Bethesda, MD 20892-1088, USA

Summary

Achieving maximal cytoreduction during surgery is a critical prognostic factor for women with advanced stage ovarian cancer. Targeting optical imaging agents directly to ovarian cancer cells by attaching them to galactosyl (galactosamine-conjugated) serum albumin, whose sugar residues bind surface lectins that are expressed in certain ovarian adenocarcinomas, may improve metastatic tumor identification and resection. Thus, we sought to demonstrate that galactosyl serum albumin-conjugated fluorophores would be a robust mechanism through which to target ovarian cancer by evaluating its tumor-targeting capability in nine human ovarian adenocarcinoma cell lines. The optical fluorophore Rhodamine Green was conjugated to galactosyl serum albumin, a non-immunogenic targeting molecule. Galactosyl serum albumin-Rhodamine Green's ability to target nine human ovarian adenocarcinoma cell lines was evaluated by flow cytometry, fluorescence microscopy, and *in vivo* optical fluorescence imaging using female athymic nu/nu mice. All nine cell lines tested bound galactosyl serum albumin-Rhodamine Green more effectively than non-glycosylated controls ($p < 0.0001$). Fluorescence microscopy demonstrated that galactosyl serum albumin-Rhodamine Green was internalized into each cell line in a galactosamine-dependent manner. *In vivo* optical fluorescence images of intraperitoneal tumor-bearing mice acquired three hours after intraperitoneal injection of galactosyl serum albumin-Rhodamine Green successfully differentiated between tumor and normal tissue. This technique also allowed the visualization of submillimeter-sized ovarian tumor implants. These results indicate that galactosyl serum albumin-Rhodamine Green can selectively target a variety of human ovarian adenocarcinomas for optical fluorescence imaging and thus may improve intraoperative tumor detection and resection.

Introduction

The majority of women with ovarian cancer have a poor prognosis because they already have advanced stage disease with peritoneal metastasis at the time of diagnosis.⁽¹⁾ Cytoreductive surgery followed by systemic or peritoneal chemotherapy is the standard of care for the initial management of women with advanced stage ovarian cancer. Optimal

* Address for correspondence: Hisataka Kobayashi, M. D., Ph. D., Molecular Imaging Program, 10 Center Drive, Bethesda, MD 20892-1088, Phone: (301) 451-4220, Fax: (301) 402-3191, Email: kobayash@mail.nih.gov.

tumor debulking, defined as having removed all lesions >1 cm in diameter, is an important surgical objective and critical prognostic factor for patients with ovarian cancer.(2) Moreover, reducing the amount of residual disease after cytoreduction to microscopic levels further improves clinical outcomes.(3,4) However, cytoreduction is often suboptimal due to the small size and location of the implants and the poor visual contrast between tumor and normal tissue under the white light conditions typically present in the operating room. One possible approach to improve the resection of tumor implants is to administer a targeted optical fluorophore that binds to ovarian tumors with high affinity and specificity. This type of imaging agent could provide the surgeon with a highly sensitive and specific intraoperative guide to tumor location that could also identify small peritoneal implants (<1 mm) that would otherwise go undetected by the unaided eye.(5)

The β -D-galactose receptor is a surface lectin that is expressed on a variety of cancer cells, including certain types of ovarian cancers.(6,7) This receptor functions to bind and internalize glycosylated proteins that contain specific sugar residues.(8) Serum albumin can be converted into galactosyl serum albumin (GSA), a ligand for this receptor, through conjugation to lectin-binding galactosamine residues.(9) Thus, a GSA-conjugated optical fluorophore may be a clinically-useful agent for use during cytoreductive surgery to highlight ovarian tumor implants.(9) Herein, we demonstrate the feasibility of this approach in a wide variety of ovarian adenocarcinomas both *in vitro* and *in vivo*.

Materials and Methods

Synthesis of galactosyl serum albumin (GSA)-Rhodamine Green (RhodG) and bovine serum albumin (BSA)-RhodG

BSA and GSA, which has 23 galactosamine residues conjugated to each BSA, were purchased from Sigma Chemical (St. Louis, MO, USA) and amido-reactive RhodG was purchased from Molecular Probes (Eugene, OR, USA). At room temperature, 400 μ g of either GSA or BSA in 196 μ L of Na_2HPO_4 was incubated with 4 μ L of a 6 mM solution (24 nmol) of RhodG-succinimidyl ester in DMSO for 15 min. The mixture was then purified with a Sephadex G-50 Gel Filtration System (PD-10; GE Healthcare, Milwaukee, WI, USA). GSA- and BSA-RhodG samples were kept at 4°C as stock solutions.

The protein concentration of GSA- and BSA-RhodG samples was determined with a Coomassie Plus Protein Assay Reagent Kit (Pierce Biotechnology, Rockford, IL, USA) by measuring the absorption at 595 nm with a UV-Vis spectrophotometer (8453 UV-Vis Value System, Agilent Technologies, Palo Alto, CA, USA) using GSA and BSA standards, respectively. The RhodG concentration of both GSA- and BSA-RhodG samples was measured by the absorption at 504 nm using the same spectrophotometer. The number of fluorophore molecules conjugated to each BSA or GSA molecule was determined by dividing the RhodG concentration of each sample by either the BSA protein concentration or GSA protein concentration, respectively. To directly compare the fluorescent intensity of GSA-RhodG to BSA-RhodG, the number of RhodG molecules conjugated each protein was made approximately equal (~3 RhodG per BSA or GSA) by adjusting the protein concentration of each sample. Since RhodG continued to fluoresce even after conjugation with GSA or BSA, the paired samples used for *in vitro* and *in vivo* experiments produced almost the same fluorescence emission signals. Finally, in order to validate that the fluorophores were associated with the respective proteins, both conjugates were analyzed by gel-filtration using a high-performance liquid chromatography system equipped with an in-line UV detector (System Gold, Beckman Coulter, Inc., Fullerton, CA, USA) and fluorescence detector (FP2020, Jasco Inc., Easton, MD, USA) using a TSK G2000 30 cm column (TOSO Bioscience LLC, Montgomeryville, PA, USA) with 0.066 M 1 $\times$ phosphate buffered saline (PBS).

Cell culture

The following human ovarian adenocarcinoma cell lines were used: SHIN3,[^](10) SKOV3,^{*} CaOV3,^{*} A2780,[#] HEY-A8,^e OVCAR4,[&] (11) OVCAR5,[&] (11) OVCAR8,[&] (11) and IGR-OV1.[&] (12) SKOV3 was grown in McCoy's 5A medium (Quality Biological Inc., Gaithersburg, MD, USA) containing 10% fetal bovine serum (FBS) (Gibco, Gaithersburg, MD, USA) and 1% non-essential amino acids (BioWhittaker, Walkersville, MD, USA). All other cell lines were grown in RPMI 1640 medium (BioWhittaker) containing 10% fetal bovine serum (FBS) (Gibco), 0.03% L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. All cell lines were grown at 37°C in 5% CO₂.

Flow cytometry

Single-color flow cytometry was performed to evaluate whether the cell lines could bind GSA-RhodG in a galactosamine-dependent fashion. Cells from each line were incubated for 16 hours, after which they were either left untreated or had either 3 µg/mL of BSA-RhodG or GSA-RhodG added to the media. After an additional 3 hour incubation, the cells were then trypsinized and washed once with 1× PBS. Flow cytometry was performed immediately using a FACScan cytometer (Becton-Dickinson, Franklin Lakes, NJ, USA). The argon ion 488 nm laser was employed for excitation. Signals from cells were collected using a 530/30 nm band-pass filter. All data was analyzed using *CellQuest* software (Becton-Dickinson, Franklin Lakes, NJ, USA). The percentage of fluorescent-gated cells and mean fluorescent intensity (MFI) is indicated for each experimental condition.

Fluorescence microscopy

Fluorescence microscopy was performed to demonstrate that GSA-RhodG is internalized by the cells in a galactosamine-dependent fashion. Cells from each line were plated on coverglass bottom culture wells and incubated for 16 hours, after which each well was left untreated or had either 3 µg/mL of BSA-RhodG or GSA-RhodG added to the media. After an additional 3 hour incubation, the coverglass with attached cells was removed and washed once with 1× PBS. Fluorescence microscopy was performed using an Olympus BX51 microscope (Olympus America Inc., Melville, NY, USA) with excitation at 470–490 nm and emission at 515 nm long pass. Transmitted light differential interference contrast (DIC) images were also acquired. Images were acquired at 20× magnification with 2 ms exposure for DIC images and 500 ms exposure for fluorescence microscopy images.

Tumor Model

All animal experiments were carried out in compliance with the Guide for the Care and Use of Laboratory Animal Resources (1996), National Research Council, and approved by the National Cancer Institute Animal Care and Use Committee. The establishment of intraperitoneal (i.p.) tumor xenografts was attempted with each cell line by the i.p. injection of 2×10^6 cells suspended in 300 µL 1× PBS into female NIH nu/nu mice (National Cancer Institute Animal Production Facility, Frederick, MD, USA). Tumor xenografts were successfully established with the SHIN3, SKOV3, OVCAR5, and OVCAR8 cell lines. Experiments with tumor-bearing mice were performed approximately 3 weeks after cell injection.

[^]provided by Dr. S. Imai, Nara, Japan

^{*}obtained from American Type Culture Collection, Manassas, VA, USA

[#]a gift from Dr. T. Fojo, National Cancer Institute, NIH, Bethesda, MD, USA

^ea gift from Dr. G. Mills, M.D. Anderson Cancer Center, Houston, TX, USA

[&]provided by Developmental Therapeutics Program, National Cancer Institute-Frederick, NIH, Frederick, MD, USA

In vivo fluorescence imaging

Tumor-bearing mice received an i.p. injection of 25 µg of either GSA-RhodG or BSA-RhodG diluted in 300 µL of 1× PBS. The mice were sacrificed by CO₂ inhalation three hours after administration of the agent and the abdominal walls were then removed. White light photos and spectral fluorescence images of the entire exposed abdomen were obtained using the Maestro™ In Vivo Imaging System (CRi Inc., Woburn, MA, USA). A loop of bowel and mesentery from each mouse treated with GSA-RhodG was then removed and imaged separately by the same system in order to demonstrate the ability of GSA-RhodG to detect submillimeter-sized ovarian tumor foci. A band pass filter from 445–490 nm and a long pass filter over 515 nm were used for excitation and emission light, respectively. The tunable filter was automatically stepped in 10 nm increments from 500 nm to 800 nm while the camera captured images at each wavelength interval with constant exposure. Commercially available Maestro™ software with spectral unmixing algorithms was used to create the spectral fluorescence composite images (Nuance ver. 1.4, CRi Inc., Woburn, MA, USA).

A direct comparison of the fluorescent intensity of BSA-RhodG-treated and GSA-RhodG-treated tumor xenografts was made by imaging the tumor nodules side-by-side *ex vivo*. A semiquantitative analysis of the two tumor nodules was performed by drawing a region of interest (ROI) as large as each tumor to determine the fluorescence intensity using ImageJ software (<http://rsb.info.nih.gov/ij/plugins/mri-analysis.html>). The dynamic range of the fluorescent intensity in arbitrary units (a.u.) was split into 256 equal-sized bins (0–255).

Results

Flow cytometry

The percentage of fluorescent-gated cells, which corresponds to cell binding by either the BSA- or GSA-RhodG conjugate, is shown in Figure 1. After incubation with GSA-RhodG, the average percentage of fluorescent-gated cells for all cell lines analyzed was 97.7% (range: 89.5–99.8) which was significantly higher than when the cells were incubated with BSA-RhodG (mean: 20.7%, range: 7.3–32.2) ($p < 0.0001$). The average MFI for cells incubated with GSA-RhodG was 170.6 (range: 74.7–310.9) which was also significantly higher than the average MFI for cells incubated with BSA-RhodG (mean: 8.6, range: 5.5–10.9) ($p < 0.0001$). The flow cytometry data indicate that all the ovarian adenocarcinoma cell lines tested bind GSA-RhodG in a galactosamine-dependent manner, suggesting that they can be targeted for *in vivo* optical fluorescence imaging by GSA-RhodG.

Fluorescence microscopy

After incubation with 3 µg/mL of GSA-RhodG, cells from each line demonstrated intracellular fluorescent GSA molecules (Figure 2A). Although the number and intensity of the fluorescent molecules differed between cell lines, these images confirmed that GSA-RhodG is internalized after binding to the ovarian cancer cells. DIC images are shown in Figure 2 for orientation. Fluorescence microscopy images were also obtained after incubation with BSA-RhodG; however, none of these images revealed intracellular fluorescent molecules (Figure 2B).

In vivo optical fluorescence imaging

Successful i.p. tumor xenografts were established with the SHIN3, SKOV3, OVCAR5, and OVCAR8 cell lines. Tumor-bearing mice received an i.p. injection of 25 µg of either GSA-RhodG or BSA-RhodG. Side-by-side *in vivo* spectral fluorescence images of the entire exposed abdomen obtained three hours after agent injection demonstrated that GSA-RhodG produced a qualitatively higher tumor-associated fluorescence than BSA-RhodG in tumor

xenografts of SKOV3 (3A), OVCAR5 (3B), and OVCAR8 (3C). White light photos of the bowel and mesentery of SKOV3 (3D), OVCAR5 (3E), and OVCAR8 tumor-bearing mice (3F) treated with GSA-RhodG reveal minimal visible tumor implants. Spectral fluorescence composite images of the same tissue, however, were able to identify numerous submillimeter-sized tumor implants (3G–3I), thus demonstrating the ability of GSA-RhodG to locate micrometastatic disease. A semiquantitative analysis of tumor fluorescence by ImageJ software (Figure 4) showed that tumor nodules derived from SKOV3 tumor-bearing mice treated with GSA-RhodG had a higher fluorescent intensity (mean: 717) than nodules removed from mice treated with BSA-RhodG (mean: 94.4) ($p < 0.0001$). Similar results were obtained for OVCAR5 and OVCAR8 tumor-bearing mice ($p < 0.0001$ for both data sets). These data reveal that GSA-RhodG targets ovarian tumor xenografts *in vivo* more effectively than non-glycosylated BSA-RhodG.

Discussion

It was previously unknown whether the optical imaging probe GSA-RhodG could be used to specifically target a wide variety of ovarian adenocarcinomas for intraoperative identification. Thus, the goal of this study was to evaluate the ability of nine human ovarian adenocarcinoma cell lines to be targeted *in vitro* and *in vivo* by GSA-RhodG. Analysis by flow cytometry showed that GSA-RhodG bound to all ovarian adenocarcinoma cell lines tested in a galactosamine-dependent manner while fluorescence microscopy documented that these cell lines internalize GSA-RhodG but not the non-glycosylated BSA-RhodG. Moreover, tumor-targeted optical fluorescence imaging with GSA-RhodG successfully identified tumors in three new models of late stage ovarian cancer, thus extending our laboratory's pilot studies using the SHIN3 cell line [9] to a broader spectrum of ovarian adenocarcinomas. These data suggest that tumor-associated lectins are expressed in sufficient quantities on all nine cell lines tested to enable them to act as a general molecular target in ovarian adenocarcinoma for GSA-RhodG. Thus, it is likely that many, if not all, ovarian adenocarcinomas could be targeted for optical imaging using GSA-RhodG.

The administration of a tumor-targeted optical imaging probe such as GSA-RhodG could provide surgeons with an intraoperative guide to tumor location that identifies submillimeter-sized tumor foci that might otherwise go undetected because of their small size or unusual location. GSA-RhodG's favorable pharmacokinetic properties, such as rapid clearance from the peritoneal space and swift hepatic metabolism that prevents re-circulation, result in high signal-to-background ratios that make it a particularly attractive imaging probe for peritoneal carcinomatosis. Thus, it is hoped that the advancement of this technology to the clinical realm will ultimately improve survival for women with late stage ovarian cancer.

The two components of this ovarian tumor-targeted optical imaging probe, RhodG as the optical fluorophore and GSA as the targeting moiety, were selected because of their favorable biologic characteristics. The short penetration of green fluorescent dyes in tissue ordinarily limits their use to *in vitro* applications in favor of near-infrared (NIR) dyes which have a longer penetration distance. However, when the target is on the surface, such as peritoneal tumor implants, green dyes are actually preferable to NIR dyes because they are efficient fluorophores that yield an extremely bright signal (Figure 3). RhodG was previously determined to be an ideal fluorophore for this type of application because its fluorescence, instead of fading like other green fluorescent probes, actually increases in intensity after internalization by ovarian cancer cells.(13) The glycoprotein avidin is a ligand for lectins like the β -D-galactose receptor (8) and has been successfully used as a targeting moiety for the *in vivo* imaging of ovarian adenocarcinomas.(5,7) However, its immunogenicity precludes its clinical use.(14,15) Thus, in a previous study, our laboratory

evaluated GSA as an alternative to avidin because a 99m technetium-labeled analogue is already in clinical use in Japan.(16) Indeed, GSA-RhodG proved to be a more effective tumor-targeted optical probe than avidin-RhodG.(9)

There are several potential clinical questions that should be considered prior to implementing this technique in humans. First, it is well known that some patients with metastatic ovarian cancer have intra-abdominal adhesions due to prior surgeries or i.p. chemotherapy that could limit the agent's access to all tumor implants. Furthermore, many patients with advanced stage ovarian cancer have literally thousands of metastases which makes the resection of all disease, even if made visible by the use of tumor-targeted optical fluorophores, a clinical impracticality. However, this type of imaging probe could have other applications apart from those already described in this report. For example, GSA-RhodG could be administered to patients undergoing surgery for stage I/II ovarian cancer in order to rule out the presence of micrometastatic disease, ensuring proper surgical staging and therapy. Additionally, since photodynamic therapy (PDT) has been shown to have an application in the treatment of advanced stage ovarian cancer,(17,18) work is underway to exchange the optical fluorophore of the tumor-targeted probe for a photo-sensitizing agent in order to deliver ovarian tumor-targeted intraoperative adjuvant PDT. After the removal of bulky metastases, a GSA-directed PDT agent could be administered that would be selectively internalized by ovarian cancer cells. Then, before closing the abdomen, the surgeon would shine an activating light on common sites of metastasis and organ surfaces in order to induce the tumor-targeted agent to produce reactive oxygen species (ROS). The linkage to GSA would be anticipated to increase both the sensitivity and specificity of the photo-sensitizer, thereby reducing collateral damage to normal tissue produced by PDT. Thus, this tumor-targeting system could provide ovarian cancer patients with an additional strategy for the management of unresected tumor implants while minimizing side effects.

A limitation of this study was that we evaluated only 9 cultured cell lines *in vitro* and induced *in vivo* tumor models using only 4 cell lines among them but not any clinical specimens. It is possible that other cell lines of ovarian cancer might produce negative results. The successful operation of this system necessitates not only binding but also internalization of GSA into the cancer cells. Therefore, we could investigate this method only with living cells or tumors but not fixed pathologic specimens. In the next step toward the clinical application, we may have to find appropriate ways to validate this method using freshly obtained surgical samples of ovarian cancer for either *in vitro* or *in vivo* animal studies.

In this report, we have demonstrated that GSA-RhodG can target a wide variety of human ovarian adenocarcinomas for optical fluorescence imaging. Future studies will focus on translating these findings to the clinical setting. It is hoped that the culmination of this work will be a clinically-useful targeted optical fluorophore that will be able to label metastatic ovarian adenocarcinoma implants for surgical resection; thereby, improving the completeness of cytoreductive surgery and leading to improved patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research.

References

1. Chan JK, Cheung MK, Husain A, et al. Patterns and progress in ovarian cancer over 14 years. *Obstet Gynecol.* 2006; 108(3 Pt 1):521–8. [PubMed: 16946210]
2. Tingulstad S, Skjeldestad FE, Halvorsen TB, Hagen B. Survival and prognostic factors in patients with ovarian cancer. *Obstet Gynecol.* 2003; 101(5 Pt 1):885–91. [PubMed: 12738145]
3. Griffiths CT. Surgical resection of tumor bulk in the primary treatment of ovarian carcinoma. *Natl Cancer Inst Monogr.* 1975; 42:101–4. [PubMed: 1234624]
4. Chi DS, Eisenhauer EL, Lang J, et al. What is the optimal goal of primary cytoreductive surgery for bulky stage IIIC epithelial ovarian carcinoma (EOC)? *Gynecol Oncol.* 2006; 103(2):559–64. [PubMed: 16714056]
5. Hama Y, Urano Y, Koyama Y, et al. In vivo spectral fluorescence imaging of submillimeter peritoneal cancer implants using a lectin-targeted optical agent. *Neoplasia.* 2006; 8(7):607–12. [PubMed: 16867223]
6. Yao Z, Zhang M, Sakahara H, Saga T, Arano Y, Konishi J. Avidin targeting of intraperitoneal tumor xenografts. *J Natl Cancer Inst.* 1998; 90(1):25–9. [PubMed: 9428779]
7. Hama Y, Urano Y, Koyama Y, Choyke PL, Kobayashi H. Targeted optical imaging of cancer cells using lectin-binding BODIPY conjugated avidin. *Biochem Biophys Res Commun.* 2006; 348(3):807–13. [PubMed: 16904640]
8. Stockert RJ. The asialoglycoprotein receptor: relationships between structure, function, and expression. *Physiol Rev.* 1995; 75(3):591–609. [PubMed: 7624395]
9. Hama Y, Urano Y, Koyama Y, Choyke PL, Kobayashi H. D-galactose receptor-targeted *in vivo* spectral fluorescence imaging of peritoneal metastasis using galactosamine-conjugated serum albumin-Rhodamine Green. *J Biomed Opt.* 2007 in press.
10. Imai S, Kiyozuka Y, Maeda H, Noda T, Hosick HL. Establishment and characterization of a human ovarian serous cystadenocarcinoma cell line that produces the tumor markers CA-125 and tissue polypeptide antigen. *Oncology.* 1990; 47(2):177–84. [PubMed: 2314830]
11. Hamilton T, Young R, Ozols R. Experimental model systems of ovarian cancer: applications to the design and evaluation of new treatment approaches. *Semin Oncol.* 1984; 11:285–98. [PubMed: 6385258]
12. Bernard J, Da Silva J, De Blois M, et al. Characterization of a human ovarian adenocarcinoma line, IGR-OV1, in tissue culture and in nude mice. *Cancer Res.* 1985; 45:4970–9. [PubMed: 3861241]
13. Hama Y, Urano Y, Koyama Y, Bernardo M, Choyke PL, Kobayashi H. A comparison of the emission efficiency of four common green fluorescence dyes after internalization into cancer cells. *Bioconjug Chem.* 2006; 17(6):1426–31. [PubMed: 17105220]
14. Hytonen VP, Laitinen OH, Grapputo A, et al. Characterization of poultry egg-white avidins and their potential as a tool in pretargeting cancer treatment. *Biochem J.* 2003; 372(Pt 1):219–225. [PubMed: 12558501]
15. Paganelli G, Chinol M, Maggiolo M, et al. The three-step pretargeting approach reduces the human anti-mouse antibody response in patients submitted to radioimmunoscinigraphy and radioimmunotherapy. *Eur J Nucl Med.* 1997; 24(3):350–1. [PubMed: 9143478]
16. Kwon AH, Matsui Y, Ha-Kawa SK, Kamiyama Y. Functional hepatic volume measured by technetium-99m-galactosyl-human serum albumin liver scintigraphy: comparison between hepatocyte volume and liver volume by computed tomography. *Am J Gastroenterol.* 2001; 96(2):541–6. [PubMed: 11232703]
17. DeLaney TF, Sindelar WF, Tochner Z, et al. Phase I study of debulking surgery and photodynamic therapy for disseminated intraperitoneal tumors. *Int J Radiat Oncol Biol Phys.* 1993; 25(3):445–57. [PubMed: 8436523]
18. Sindelar WF, DeLaney TF, Tochner Z, et al. Technique of photodynamic therapy for disseminated intraperitoneal malignant neoplasms. Phase I study. *Arch Surg.* 1991; 126(3):318–24. [PubMed: 1998474]

Figure 1.

Flow cytometry results from nine human ovarian adenocarcinoma cell lines after incubation with either 3 $\mu\text{g}/\text{mL}$ of GSA-RhodG (green lines) or BSA-RhodG (purple lines). Untreated controls (black lines) are also shown. The percentage of fluorescent-gated cells, which corresponds to the number of cells that were labeled by the protein-fluorophore conjugates, is shown for each experimental condition. The percentage of fluorescent-gated cells and MFI is highest after GSA-RhodG incubation for all nine cell lines.

Figure 2.

Transmitted light differential interference contrast (DIC) (left images) and fluorescence microscopy images (right images) from nine human ovarian adenocarcinoma cell lines after incubation with 3 $\mu\text{g}/\text{mL}$ of GSA-RhodG (A) or BSA-RhodG (B). The appearance of intracellular fluorescent GSA molecules demonstrates that only GSA-RhodG is internalized by the cells.

Figure 3.

Figure 3A, Side-by-side spectral fluorescence composite image of the exposed abdomens of SKOV3 tumor-bearing mice treated with GSA-RhodG (left) and BSA-RhodG (right) demonstrates brighter tumor-associated fluorescence with GSA-RhodG than with BSA-RhodG. Figures 3B and 3C reveal similar results for OVCAR5 and OVCAR8 tumor-bearing mice, respectively. Figure 3D, White light photo of a loop of bowel and mesentery from a SKOV3 tumor-bearing mouse treated with GSA-RhodG identifies only one tumor nodule (yellow arrow). Figures 3E and 3F reveal similar results for OVCAR5 and OVCAR8 tumor-bearing mice, respectively. Figure 3G, Spectral fluorescence composite image of the same tissue shown in 3D now reveals multiple tumor nodules (green) indicating that targeted optical imaging can identify more disease than the unaided eye. Figures 3H and 3I reveal similar results for OVCAR5 and OVCAR8 tumor-bearing mice, respectively. (Note: red = autofluorescence of gastrointestinal tract)

Figure 4.

Top, Side-by-side *ex vivo* fluorescent image of tumor nodules derived from SKOV3 tumor-bearing mice treated with GSA-RhodG (left) and BSA-RhodG (right). Using ImageJ software, a region of interest was drawn around each nodule and their fluorescent intensity plotted on a histogram (bottom). Administration of GSA-RhodG resulted in higher fluorescent intensity (green line) in tumors than did BSA-RhodG administration (purple line) ($p < 0.0001$). Although not shown, similar results were obtained with nodules derived from OVCAR5 and OVCAR8.