Remodeling of the Tumor Microenvironment by Combined Treatment with a Novel Radiosensitizer, α-Sulfoquinovosylmonoacylglycerol (α-SQMG) and X-irradiation

KEISUKE OHTA[1,2], HIROSHI MURATA[2,3], YOKO MORI[1], MASAHIRO ISHIMA[1,2], FUMIO SUGAWARA[3], KENGO SAKAGUCHI[3] and MASAHIKO MIURA[1]

[1]*Oral Radiation Oncology, Department of Oral Restitution, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8549, Japan;*
[2]*CANGO Corp., Sinjuku-ku, Tokyo, Japan;*
[3]*Department of Applied Biology, Faculty of Science and Technology, Tokyo University of Science, Noda, Chiba 278-8510, Japan*

Abstract. *Aim: The purpose of this study was to examine the effects of combined treatment with a specific type of sulfoglycolipid, α-sulfoquinovosylmonoacylglycerol (α-SQMG), and X-irradiation (XRT) on the remodeling of tumor microenvironments. Materials and Methods: A human colon cancer cell line, SW480, was used in this study. The cells were injected subcutaneously into nude mice and the resulting tumors were treated with α-SQMG that was injected intravenously and/or X-irradiation (XRT). The tumor volumes were monitored and the microenvironments of the treated tumors were immunohistochemically analyzed for angiogenesis, pericyte recruitment, and hypoxic fractions using markers for CD31, collagen IV, α-Sma, and pimonidazole. Results: The combined treatment with α-SQMG (five daily injections from days zero to four) and X-irradiation (two fractions on days zero and three) synergistically enhanced the radioresponse of tumor growth in vivo, whereas α-SQMG treatment alone had no effect. The tumor vessel density was significantly decreased at days 10 and 20 after initiating the combined treatment. On day 20, areas with overlapping CD31 and collagen IV expression were rarely observed, suggesting that the normal structures of most tumor vessels had collapsed. α-Sma staining was significantly increased and pimonidazole staining was significantly reduced at 24 and 72 h, but not 6 h, after the first combined treatment. Conclusion: The combined treatment induced remodeling of the microenvironments in SW480 tumors, which might contribute to the radiosensitization to the second irradiation.*

Correspondence to: Masahiko Miura, Oral Radiation Oncology, Department of Oral Restitution, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan. Tel/Fax: +81 358035897, e-mail: masa.mdth@tmd.ac.jp

Key Words: Vascular normalization, pericytes, angiogenesis, radiotherapy, hypoxia, tumor microenvironment, α-SQMG.

Angiogenesis is an essential process for solid tumor growth, which has led to heightened interest in antiangiogenic therapies (1) and the development of agents that target proangiogenic factors (2-5). However, monotherapy for tumor angiogenesis is insufficient to completely eradicate solid human tumors. Furthermore, thrombotic or hemorrhagic complications and hypertension are induced after prolonged use of antiangiogenic therapies (6). However, previous studies have shown that the combined use of antiangiogenic agents and chemotherapeutic agents or irradiation is efficacious in pre-clinical and clinical settings (7-9).

Jain *et al*. reported that antiangiogenic treatment temporarily induces the remodeling of tumor microenvironments. Furthermore, an increase in pericytes surrounding the tumor vessels decreases vessel permeability, which improves tumor swelling. This remodeling of the tumor vessels decreases the interstitial fluid pressure, and subsequently increases the pressure inside the vessels. Finally, the diffusion efficiency of small molecules and oxygen increases (10-12) through a phenomenon known as 'vascular normalization'. As long as this temporal phenomenon occurs, the efficacy of combined therapies that include antiangiogenic agents and chemotherapy or radiotherapy is enhanced. However, this change does not persist, and the vessel density is reduced over time, consequently leading to hypoxic conditions (10, 11), and there are many independent studies that support this notion (13, 14).

It was previously reported that mono acyl-forms of sulfoquinovosylacylglycerol (SQAG), α-sulfoquinovosylmonoacylglycerol (α-SQMG), which were originally derived from sea urchins (15), higher plants (16), or sea algae (17), are able to enhance the radioresponse in solid human tumors when combined with two fractions of X-irradiation (XRT) (18). Interestingly, drug treatment alone did not affect tumor growth. It was further determined that the target of the combined treatment appeared to be tumor angiogenesis rather than the tumor cells (18), which could explain the enhanced radioresponse. However, the precise mechanism of the enhanced tumor radioresponse is largely unknown. It was hypothesized that vascular normalization could lead to the enhanced radioresponse in our combined regimen. In this study, several lines of evidence are provided that this combination therapy, but not each individual treatment, leads to vascular normalization.

Materials and Methods

Cell lines and animal studies. A human colon adenocarcinoma cell line, SW480, was obtained from the Cell Resource Center for Biomedical Research (Sendai, Japan) and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1 mM sodium pyruvate at 37˚C in a 5% CO_2 humidified atmosphere. *In vivo* studies were performed in accordance with the Guidelines for Animal Experimentation of Tokyo Medical and Dental University. Viable tumor cells (2×10^6) were implanted subcutaneously into the right hind legs of male KSN nude mice (6-8 weeks old). After the tumor volume reached 100 to 300 mm^3, the tumors were treated and the tumor growth was monitored by palpation. The size of the palpable tumors was measured using calipers every two days. The tumor volume (V, mm^3) was estimated based on the length (mm) × width (mm) × height (mm) × π/6.

Treatments. Synthesized α-SQMG (19, 20) was prepared one day before experimentation, and dissolved in saline at the appropriate concentrations. To analyze the tumor radioresponse, 2 mg/kg α-SQMG (dissolved in 100 μl of saline) was administered intravenously to the 'α-SQMG' group and 'XRT+α-SQMG' group (administered five times daily from days zero to four). The same amount of saline was injected in the control group and XRT group. Non-anesthetized mice were irradiated (6 Gy/fraction) on days zero and three with X-ray therapeutic machines HS-225 (225 kVp, 15 mA, 1.0 mm Cu filtration) (Shimadzu, Kyoto, Japan) while shielding the body with lead. Mice were sacrificed, and the tumors were dissected and rapidly frozen on days 10 and 20. To analyze vascular normalization, the same treatments were performed as for the tumor radioresponse except that the mice were irradiated and administered α-SQMG once on day zero, and 60 mg/kg pimonidazole dissolved in saline was injected intraperitoneally 30 min before the mice were sacrificed at the indicated times after starting treatment.

Immunofluorescence staining and image analysis. To analyze the tumor radioresponse, consecutive 10 μm-thick tumor cryosections were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature, incubated in blocking buffer, and then probed overnight at 4˚C with primary antibodies that are specific for endothelial cells (CD31; 1:20) (BD Pharmingen, San Diego, CA, USA) or the vascular basal membrane (collagen IV; 1:1000)(Chemicon, Temecula, CA, USA). After extensive washing in PBS, the sections were incubated with Alexa 488- or Alexa 594-conjugated secondary antibodies (Invitrogen, Carlsbad, CA, USA) for CD31 (1:200) and collagen IV (1:400), respectively, for 30 min at room temperature. To examine vascular normalization, the cryosections were fixed in 4% formaldehyde, incubated in blocking buffer, and then probed with a Cy3-conjugated antibody specific for pericytes (α-smooth muscle actin: α-Sma; 1:1000) (Sigma) overnight at 4˚C. To detect hypoxia, the sections were fixed in ice-cold acetone for 10 min and then incubated with an anti-pimonidazole primary antibody followed by an Alexa 488-conjugated secondary antibody according to the manufacturer's instructions (Hypoxyprobe-1™; Natural Pharmacia International, Burlington, MA, USA). After extensive washing in PBS, all of the immunostained sections were covered with an antifade agent containing DAPI (Vector Laboratories, Burlingame, CA, USA) and examined with a fluorescence microscope BX51 (Olympus, Tokyo, Japan).

Tumor sections were imaged using a 4× or 10× objective (Olympus), digitized after background correction, and then analyzed using Photoshop CS3 software (Adobe Systems, San Jose, CA, USA). Eight-bit grayscale image montages from adjacent microscopic fields were acquired and digitally overlaid for multiple stains. To quantify the stained areas, the grayscale images were converted to binary images, and the number of pixels was counted. The normalization index was calculated as the ratio of α-Sma to CD31. For all quantifications, the data were normalized based on the DAPI intensity, which was stained simultaneously with the specific markers.

Statistical analysis. Mean values were statistically compared using Student's *t*-test, and differences with $p<0.05$ were considered statistically significant.

Results

Combined treatment enhances growth inhibition in SW480 xenografts and reduces the tumor microvessel density. It was previously reported that combined treatment with α-SQMG and two fractions of XRT synergistically enhanced the radioresponse of tumor growth in solid tumors that were generated by subcutaneously transplanting human tumor cell lines into nude mice (18). In this study, it was demonstrated that the solid tumors derived from a human colon cancer cell line, SW480, also exhibit a similar response (Figure 1). It should be noted that α-SQMG alone did not significantly affect tumor growth. However, the radioresponse was significantly enhanced when administered in combination with α-SQMG.

Next, the treated solid tumors were examined immunohistochemically focused on tumor vessels because it has been determined that the combined treatment strongly inhibits angiogenesis (18). Dual staining for CD31 and collagen IV, which are markers for endothelial cells and the vascular basal membrane, respectively, revealed that tumor microvessel density was significantly decreased at days 10

and 20 after starting treatment (Figure 2A, 2B). On the other hand, XRT alone tended to decrease both markers, but these reductions were not significant (Figure 2B). Interestingly, both markers co-localized in most tumor vessels at day 10. However, on day 20, these areas of co-localization were rarely detected and the markers predominantly localized to separate areas (Figure 2A). These results appear to reflect the transient pathologic process in which tumor vessels collapse at later time points as a result of the combined treatment. The properties were not observed in tumors that were treated with only one of these components.

Combined treatment enhances the expression of α-Sma around the tumor microvessels in SW480 xenografts. Jain *et al*. reported that antiangiogenic treatment temporarily induces vascular normalization, leading to oxygenation of tumor tissues approximately three days after the treatment (10). In this study, it was noticed that the second irradiation in our treatment regimen was incidentally performed on day three after starting the combined treatment (Figure 1), which prompted an examination of whether vascular normalization occurred after the first combined treatment. To test this possibility, first the recruitment of pericytes around the tumor vessels at early time points after treatment was examined. Pericytes recruitment is highly characteristic of vascular normalization (10). For this purpose, the normalization index was determined as the ratio of the stained areas for α-Sma, a marker for pericytes, to those for CD31. α-Sma staining was minimally detected in both untreated samples and in treated samples at 6 h after the combined treatment. However, at 24 and 72 h after treatment, there was a remarkable increase in α-Sma staining (Figure 3A, 3B).

Combined treatment reduces the hypoxic fractions in SW480 xenografts. If vascular normalization occurs during this combination therapy, it is important to determine whether tumor oxygenation occurs at the second irradiation. For this purpose, a hypoxic marker, pimonidazole, was used. Pimonidazole was injected 30 min before sacrifice and detected with an anti-pimonidazole antibody (Figure 4A, 4B). Protein adducts of pimonidazole are formed when the tissue pO2 is below 10 mmHg, and the antibody specifically recognizes these adducts. Previous reports have shown that irradiation alone can induce oxygenation in many tumors (21). However, under the current experimental conditions, it was not possible to clearly detect oxygenation in these tumors at the indicated times. On the other hand, at 24 and 72 h but not 6 h after the combined treatment, there was a significant decrease in the hypoxic fractions, which was consistent with the kinetic increase in pericytes as described in Figure 3B. Furthermore, a micronucleus assay was performed using cytochalasin B-induced binucleated cells that were derived from treated tumors (22). The preliminary

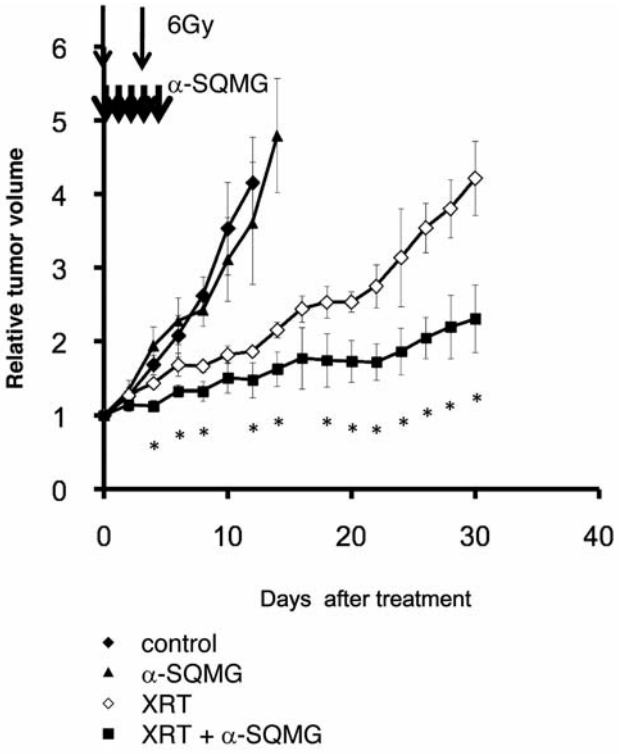
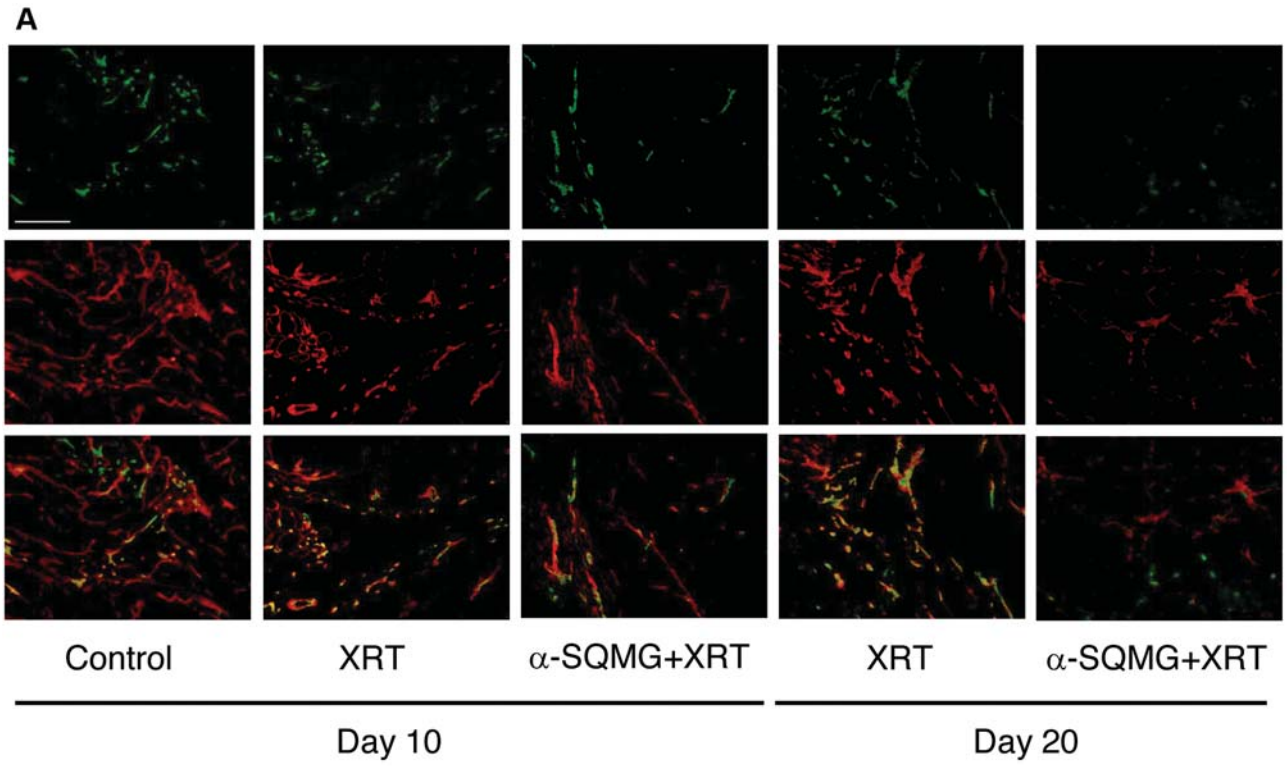


Figure 1. *Relative tumor volume as a function of the days after initiating treatment. Five injections of α-SQMG (2 mg/kg, i.v.) plus two fractions of X-irradiation (6 Gy/fraction) were administered. Vehicle, α-SQMG (2 mg/kg/injection), XRT (6 Gy/fraction), and α-SQMG and XRT. Arrows indicate injections. Each point represents the mean±SD of three or four tumors. *p<0.05: XRT vs. XRT+α-SQMG.*

data showed that the micronuclei yield was significantly increased (approximately two-fold) in tumors that received 12 Gy at 72 h compared to 6 h after the combined treatment. This finding supports the importance of the second irradiation for radiosensitization in our combined regimen.

Discussion

Previous results indicated that antiangiogenic effects contribute to the enhanced radioresponse in xenograft tumors after they are treated with a combination of a novel sulfoglycolipid, α-SQMG, and two fractions of XRT (8). There is accumulating evidence that vascular normalization occurs at the early stages in the antiangiogenic process and reduces hypoxic fractions (10), which prompted the testing of the possibility that there is radiosensitization at the time of the second irradiation in the combined regimen. This study demonstrated that the tumors treated with the combined regimen have characteristic properties of vascular normalization, such as pericyte recruitment and reduced hypoxic fractions, after approximately three days, which was



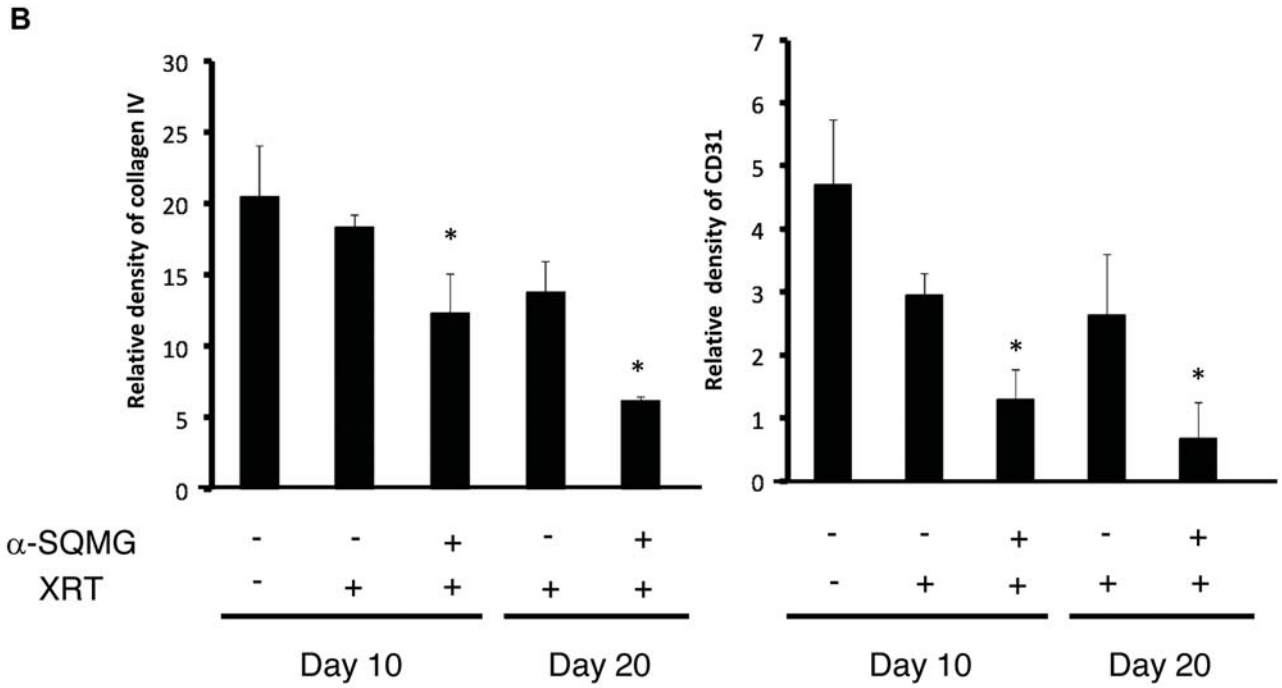


Figure 2. *Effects of α-SQMG and/or X-irradiation (XRT) on tumor vessels in SW480 xenografts. A: Representative images of CD31 and collagen IV staining in treated tumors on days 10 and 20 after initiating treatment. The control sample was prepared 10 days after the treatment was initiated in the treatment groups. Upper panel, CD31; middle panel, collagen IV; lower panel, merged images. Bar, 200 μm. B: Quantitative comparisons of collagen IV and CD31 on day 10 or 20 after initiating treatment. Each bar represents the mean±SD of three different tumors. The mean of approximately 10 fields near the center of each tumor was assigned a representative value for each tumor. The relative density was normalized to the DAPI intensity. *p<0.05 vs. control.*

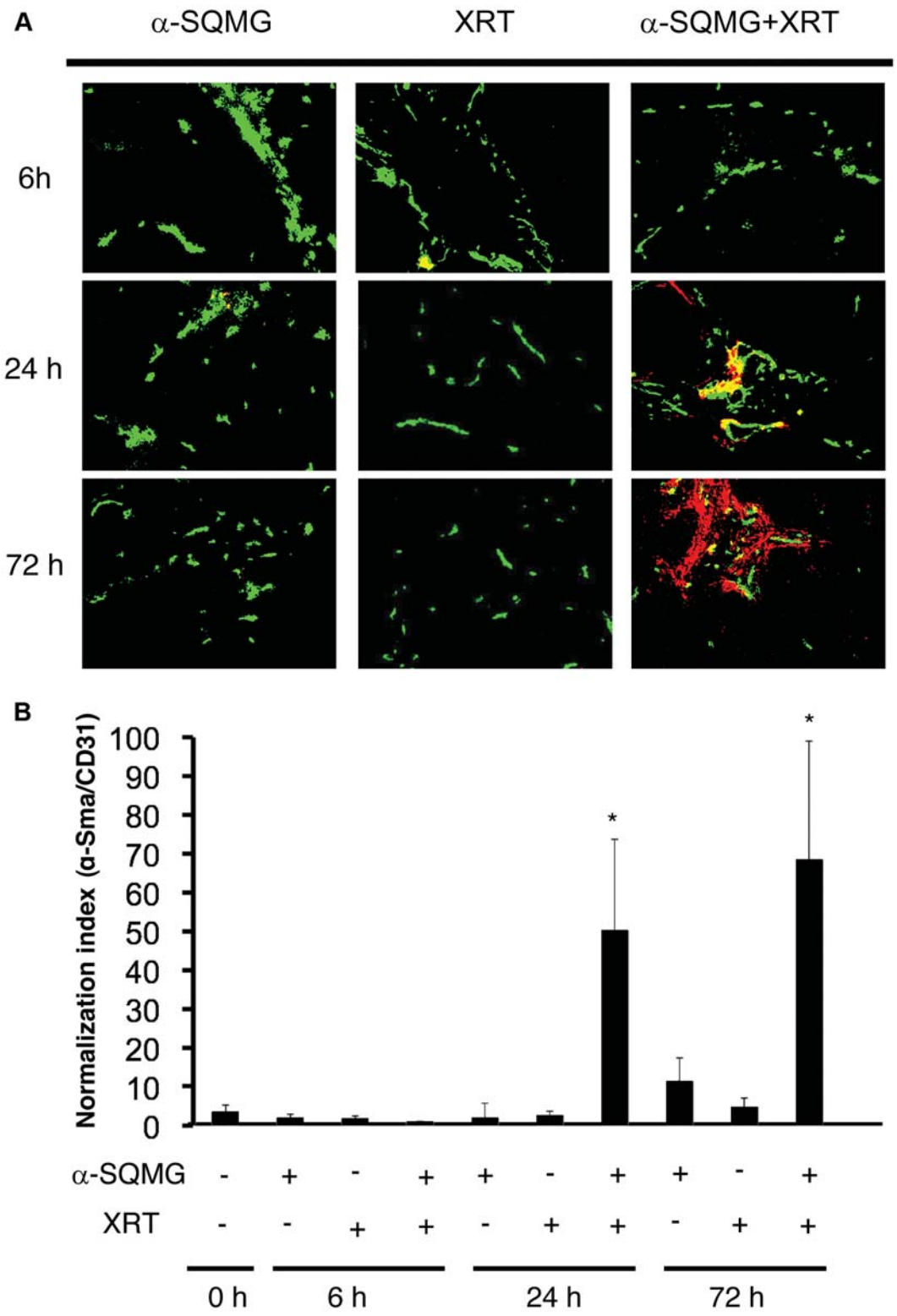


Figure 3. *Effects of α-SQMG and/or X-irradiation (XRT) on CD31 and α-Sma in SW480 xenografts. A: Representative images of pericytes (red: α-Sma), microvessels (CD31: green), and microvessels covered by pericytes (yellow) at 6, 24 or 72 h after treatment. Merged images are presented. Bar, 200 μm. B: Quantitative comparisons of the normalization index (α-Sma:CD31 ratio) at the indicated times after treatment. Each bar represents the mean±SD of three different tumors. The mean of approximately 10 fields taken from the center of each tumor was assigned a representative value for each tumor. The ratio was normalized to the DAPI intensity. *p<0.05 vs. control.*

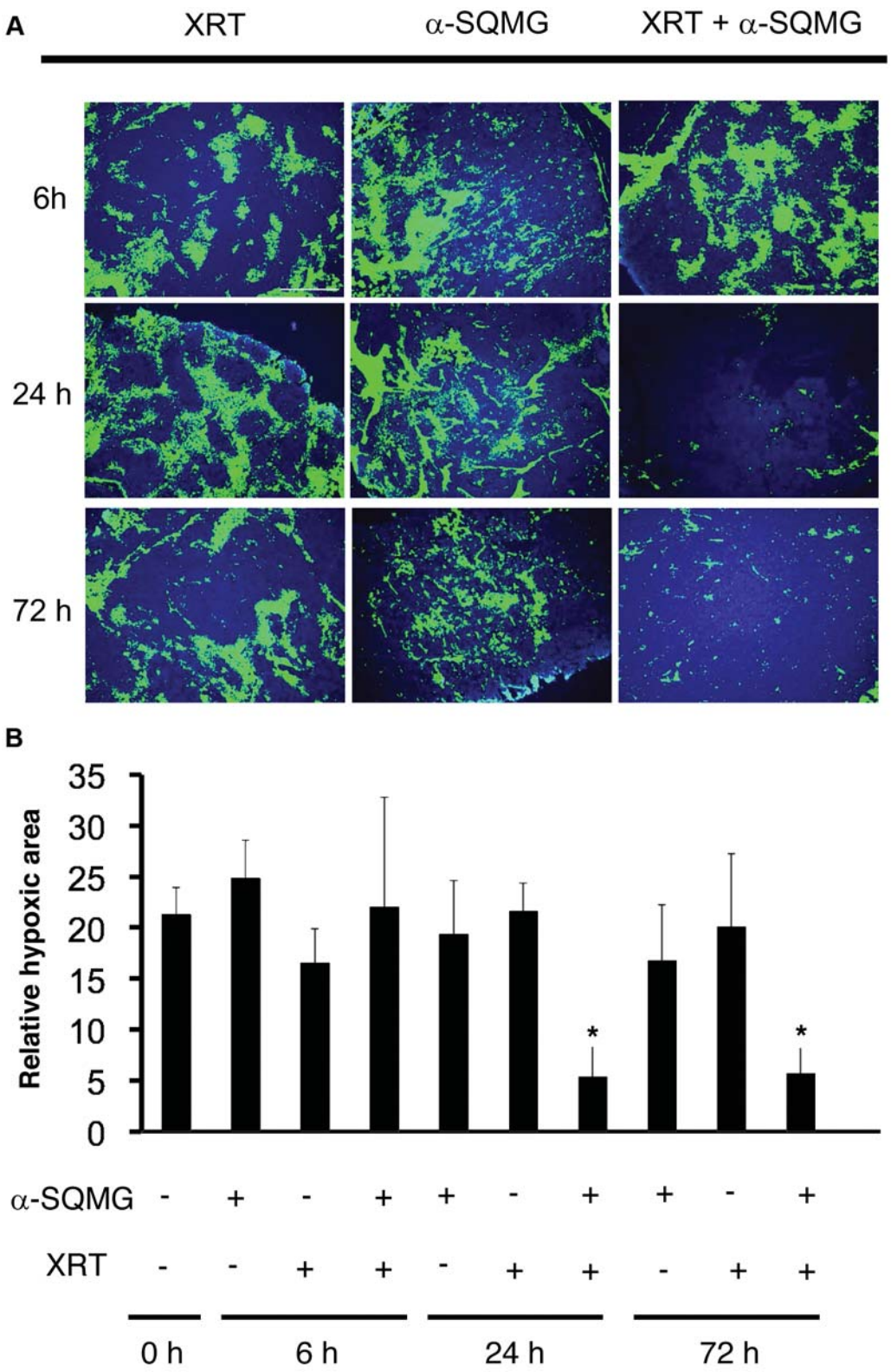


Figure 4. *Effects of α-SQMG and/or X-irradiation (XRT) on the hypoxic fractions in SW480 xenografts. A: Representative images of hypoxic fractions (green) at 6, 24 or 72 h after treatment. The blue backgrounds represent the DAPI-positive areas. Bar, 1 mm. B: Quantitative comparisons of hypoxic fractions at 6, 24, and 72 h after treatment. Each bar represents the mean±SD of three different tumors. The mean of approximately four fields taken near the center of each tumor was assigned a representative value for each tumor. The ratio was normalized to the DAPI intensity. *p<0.05 vs. control.*

when the second irradiation was administered. These findings strongly suggest that remodeling of the tumor microenvironment also contributes to the enhanced radioresponse.

These findings illustrate that a two step-mechanism underlies the enhanced radioresponse in this treatment regimen. The first step is the radiosensitization of oxygenated tumor tissues to the second irradiation due to vascular normalization, and this process occurs in the early stages after treatment. The second step is the inhibition of angiogenesis, which increases the hypoxic fractions and helps reduce tumor growth. This regimen would be ideal if only two fractions of irradiation are administered. However, conventional radiotherapy consists of ~30 fractions, and thus the radioresponse that occurs after hypoxia is induced is rather inefficient. Similar situations may also occur in combination therapies containing chemotherapy. Current clinical reports have indicated that a combination of antiangiogenic agents and chemotherapeutic agents are unlikely to be highly effective (8, 9, 23), which may be attributed to insufficient optimization and timing of the combined therapy. As Jain *et al*. has implicated, vascular normalization likely has a limited time window (10). Thus, it will be highly important to screen and identify biomarkers that can be used to monitor vascular normalization. As the mechanism of vascular normalization is still largely unknown, it is also possible that elucidating this mechanism will help determine the optimal window, which would potentially improve the timing for combined therapies. Furthermore, it is highly important that the molecular mechanism underlying the enhanced radioresponse of α-SQMG is elucidated.

Another characteristic of α-SQMG is that the agent alone has no substantial effects on tumor growth at low concentrations, but induces radiosensitizing effects. By definition, agents with this property are called radiosensitizers (24). These types of agents could remarkably reduce adverse effects because they are typically injected systemically. Recent technology in the field of radiation oncology that concentrates the radiation on tumor tissues will further enhance the usefulness of this agent.

In conclusion, although α-SQMG has great potential for radiosensitization, including its ability to remodel the tumor microenvironment, such as vascular normalization, and reduce adverse effects, there are still problems that need to be resolved before this therapy is clinically implemented. Multidisciplinary resolutions are required to determine the optimal combinations and the most efficient outcomes.

Acknowledgements

This study was supported in part by the Program for Promotion of Fundamental Studies of Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

References

1 Folkman J: Tumor angiogenesis: therapeutic implications. New Engl J Med *285*: 1182-1186, 1971.

2 O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH and Folkman J: Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell *79*: 315-328, 1994.

3 O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR and Folkman J: Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell *88*: 277-285, 1997.

4 Fong TA, Shawver LK, Sun L, Tang C, App H, Powell TJ, Kim YH, Schreck R, Wang X, Risau W, Ullrich A, Hirth KP and McMahon G: SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. Cancer Res *59*: 99-106, 1999.

5 Ferrara N: Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Semin Oncol *29(Suppl 16)*: 10-14, 2002.

6 O'Reilly MS: Targeting multiple biological pathways as a strategy to improve the treatment of cancer. Clin Cancer Res *8*: 3309-3310, 2002.

7 Mauceri HJ, Hanna NN, Beckett MA, Gorski DH, Staba MJ, Stellato KA, Bigelow K, Heimann R, Gately S, Dhanabal M, Soff GA, Sukhatme VP, Kufe DW and Weichselbaum RR: Combined effects of angiostatin and ionizing radiation in antitumor therapy. Nature *394*: 287-291, 1998.

8 Vincenzi B, Santini D, Russo A, Spoto C, Venditti O, Gasparro S, Rizzo S, Zobel BB, Caricato M, Valeri S, Coppola R and Tonini G: Bevacizumab in association with de Gramont 5-fluorouracil/folinic acid in patients with oxaliplatin-, irinotecan-, and cetuximab-refractory colorectal cancer: a single-center phase 2 trial. Cancer *115*: 4849-4856, 2009.

9 Hsu CH, Yang TS, Hsu C, Toh HC, Epstein RJ, Hsiao LT, Chen PJ, Lin ZZ, Chao TY and Cheng AL: Efficacy and tolerability of bevacizumab plus capecitabine as first-line therapy in patients with advanced hepatocellular carcinoma. Br J Cancer *102*: 981-986, 2010.

10 Jain RK: Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science *307*: 58-62, 2005.

11 Jain RK, Duda DG, Clark JW and Loeffler JS: Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol *3*: 24-40, 2006.

12 Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ and Jain RK: Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res *64*: 3731-3736, 2004.

13 Helfrich I, Scheffrahn I, Bartling S, Weis J, von Felbert V, Middleton M, Kato M, Ergün S and Schadendorf D: Resistance to antiangiogenic therapy is directed by vascular phenotype, vessel stabilization, and maturation in malignant melanoma. J Exp Med *207*: 491-503, 2010.

14 McGee MC, Hamner JB, Williams RF, Rosati SF, Sims TL, Ng CY, Gaber MW, Calabrese C, Wu J, Nathwani AC, Duntsch C, Merchant TE and Davidoff AM: Improved intratumoral oxygenation through vascular normalization increases glioma sensitivity to ionizing radiation. Int J Radiat Oncol Biol Phys *76*: 1537-1545, 2010.

15 Sahara H, Ishikawa M, Takahashi N, Ohtani S, Sato N, Gasa S, Akino T and Kikuchi K: *In vivo* anti-tumour effect of 3'-sulphonoquinovosyl 1'-monoacylglyceride isolated from sea urchin *(Strongylocentrotus intermedius)* intestine. Br J Cancer *75*: 324-332, 1997.

16 Mizushina Y, Watanabe I, Ohta K, Takemura M, Sahara H, Takahashi N, Gasa S, Sugawara F, Matsukage A, Yoshida S and Sakaguchi K: Studies on inhibitors of mammalian DNA polymerase alpha and beta: sulfolipids from a pteridophyte, *Athyrium niponicum*. Biochem Pharmacol *55*: 537-5341, 1998.

17 Ohta K, Mizushina Y, Hirata N, Takemura M, Sugawara F, Matsukage A, Yoshida, F and Sakaguchi K: Sulfoquinovosyl-diacylglycerol, KM043, a new potent inhibitor of eukaryotic DNA polymerases and HIV-reverse transcriptase type 1 from a marine red alga, *Gigartina tenella*. Chem Pharm Bull (Tokyo) *46*: 684-686, 1998.

18 Sakimoto I, Ohta K, Yamazaki T, Ohtani S, Sahara H, Sugawara F, Sakaguchi K and Miura M: α-Sulfoquinovosylmonoacylglycerol is a novel potent radiosensitizer targeting tumor angiogenesis. Cancer Res *66*: 2287-2295, 2006.

19 Sahara H, Hanashima S, Yamazaki T, Takahashi S, Sugawara F, Ohtani S, Ishikawa M, Mizushina Y, Ohta K, Shimozawa K, Gasa S, Jimbow K, Sakaguchi K, Sato N and Takahashi N: Anti-tumor effect of chemically synthesized sulfolipids based on sea urchin's natural sulfonoquinovosylmonoacylglycerols. Jpn J Cancer Res *93*: 85-92, 2002.

20 Murakami C, Yamazaki T, Hanashima S, Takahashi S, Takemura M, Yoshida S, Ohta K, Yoshida H, Sugawara F, Sakaguchi K and Mizushina Y: A novel DNA polymerase inhibitor and a potent apoptosis inducer: 2-mono-*O*-acyl-3-*O*-(alpha-D-sulfoquinovosyl)-glyceride with stearic acid. Biochim Biophys Acta *1645*: 72-80, 2003.

21 Hall EJ and Giaccia AJ: Oxygen effect and reoxygenation. *In*: Radiobiology for the Radiologist, 6th edn. Hall EJ and Giaccia AJ (eds.). Philadelphia, Lippincott Williams & Wilkins, pp. 85-105, 2006.

22 Masunaga S, Ono K, Wandl EO, Fushiki M and Abe M: Use of the micronucleus assay for the selective detection of radiosensitivity in BUdR-unincorporated cells after pulse-labelling of exponentially growing tumour cells. Int J Radiat Biol *58*: 303-311, 1990.

23 Kindler HL, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, Wozniak TF, Picus J, Bhargava P, Mayer RJ and Schilsky RL and Goldberg RM: Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). J Clin Oncol *28*: 3617-3622, 2010.

24 Hall EJ and Giaccia AJ: Radiosensitizers and bioreductive drugs. *In*: Radiobiology for the Radiologist, 6th edn. Hall EJ and Giaccia AJ (eds.). Philadelphia, Lippincott Williams & Wilkins, pp. 419-431, 2006.

Received September 18, 2010
Revised October 8, 2010
Accepted October 11, 2010