Mechanism of Photofrin-enhanced Ultrasound-induced Human Glioma Cell Death

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Abstract. *Background: Low-intensity ultrasound showed tumor cell killing by a non-thermal effect in human leukemia cells. The aim of our study was to investigate the efficacy of low-intensity ultrasound on malignant astrocytic tumor cells with the photosensitizer, Photofrin, which is taken up by the cell surface receptor, low density lipoprotein receptor-related protein/α2-macroglobulin receptor (LRP/α2MR). Materials and Methods: Cells were sonicated with continuous wave ultrasound with or without the presence of Photofrin (75 mg/ml) at an intensity of 0.3 W/cm^2 for a duration of 5, 15, or 30 s. Results: Ultrasound alone induced instant cell killing immediately after sonication in both U251MG and U105MG malignant gliomas cells. In U251MG cells, which expressed LRP/α2MR, significant enhancement of cell killing was observed following Photofrin pretreatment, 52.7±17.5%, 13.0±4.6% and 3.9±0.9% for 5, 15, and 30 s respectively ($p<0.05$). This enhancement of cell killing was abolished by preincubation with receptor-associated protein (RAP) which binds specifically to LRP/α2MR. This enhancement by Photofrin was not achieved in U105MG which did not express LRP/α2MR. U251MG cells accumulated 2.43±0.25 Photofrin mg/mg protein, which significantly decreased with RAP pretreatment (1.38±0.22 Photofrin mg/mg protein) ($p<0.05$). U105MG cells accumulated 1.31±0.16 Photofrin mg/mg protein, which was significantly less than in U251MG cells. Photofrin uptake was not altered by RAP pretreatment in U105MG cells. U251MG cells exposed to ultrasound in the presence of Photofrin showed multiple surface pores and dimple-like craters. Conclusion: This is the first report to demonstrate the usefulness of low-intensity ultrasound for the cell killing of malignant glioma cells. Antitumor activity might be enhanced by combination with photosensitizer, which is transported by cell surface LRP/α2-MR to some degree.*

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Malignant glial tumors comprise the majority of primary intracranial tumors and aggressively invade surrounding normal brain tissues (1). A variety of multimodal local adjuvant therapies, including implantation of lomustine-releasing wafers (2, 3), interstitial brachytherapy (4), and radiosurgery boost (5), have been used to treat malignant gliomas. Unfortunately, there has been only little improvement in the outcome for patients with malignant glioma after these therapies (6, 7). A more aggressive local therapy is required to eradicate unresectable "nests" of tumor cells invading adjacent normal brain tissue.

Photodynamic therapy (PDT) with laser light of an appropriate wavelength has been extensively investigated in laboratory studies for the treatment of a variety of brain tumors, particularly gliomas, and has been applied in clinical trials (8). The efficacy of this therapy is based on the selective uptake of photosensitizer by tumor relative to the surrounding normal tissue, followed by irradiation with light to activate photosensitizer (8, 9). The main advantage of photodynamic therapy lies in its ability to select tumor cells that are infiltrating brain parenchyma in combination with photosensitizer, which causes oxidative damage to a variety of cellular targets and subsequent tumor necrosis (10, 11). Unfortunately, it is quite difficult in PDT to select the optimal individualized treatment conditions of light-dose volume and geometry, and to treat massive or deep-seated tumors due to the poor penetration of optical beams into tissues (12). This has severely compromised the curative ability of PDT.

High intensity focused ultrasound has been used in cancer therapy as a device to generate heat for thermal ablation and for hyperthermia (13, 14). Nowadays, low-level ultrasound

rather than high-intensity focused ultrasound is also attracting interest to enhance the tumor cell killing by a non-thermal effect (15-19). Nonthermal ultrasound is also a bimodal therapy with nontoxic photosensitizer in isolation which is tumoricidal in combination (15). Nonthermal ultrasound can induce cell killing by cell lysis *via* pore formation on irradiated cell membranes (16), necrosis and apoptosis (17), which were associated with the occurrence of ultrasound-induced cavitation (20). Ultrasound can penetrate intervening tissues and deliver its energy to nonsuperficial objects (21). This is a unique advantage over electromagnetic modalities, such as laser and microwave, for the noninvasive treatment of deep-seated tumors. The mechanism of intracellular uptake of photosensitizer, hematoporphyrin derivative, consists of free equilibration along a concentration gradient and receptor-mediated uptake. A simple diffusion mechanism could not explain why photosensitizing agents are preferentially taken up and retained by tumor cells (11, 22). It has been reported that low-density lipoprotein (LDL) receptor plays an important role in the transport and delivery of hydrophobic photosensitizers such as Photofrin (23, 24). Luna *et al*. demonstrated that a lack of low-density lipoprotein receptor-related protein/α2-macroglobulin receptor (LRP/α2MR) expression caused reduced intracellular uptake of Photofrin in PDT-resistant tumor cells (25).

In this study, we sought to determine if the acute cytotoxic effect of low-level ultrasound on human glioma cells is enhanced by the presence of photosensitizer, Photofrin, and if differences in enhancement of ultrasound cell killing by Photofrin could be observed in glioma cells of various phenotypes of LRP/α2MR expression, using receptor-associated protein (RAP), which specifically binds to LRP/α2MR.

Materials and Methods

Cell lines. The human malignant glioma cell lines U105MG and U251MG were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum, 20 mM L-glutamine, penicillin G (100 IU/ml) and streptomycin (100 mg/ml) in an atmosphere of 95% humidified air, and 5% CO_2 at 37˚C. U251MG was obtained from the Human Science Research Resources Bank (Osaka, Japan).

RNA Extraction and RT-PCR analysis. For RNA extraction, cells were grown in 100-mm plates to 80-90% confluence. Total RNA from each cell line was prepared from 3-5×10^7 cells using RNAzol B reagent (Tel-Test, Inc. Friendswood, TX, USA) according to the manufacturer's instructions. cDNA was synthesized from 2.5 μg of total RNA using a cDNA cycle kit (Invitrogen, San Diego, CA USA) with random hexamers. To amplify the cDNA, 0.5 μg of the reverse-transcribed cDNA for cell lines were subjected to 30 cycles of PCR in 50 μl of 1 x buffer [10 mM Tris-HCl, (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 1% gelatin and 5% dimethyl sulfoxide (DMSO)] containing 0.2 mmol/l each of dATP, dCTP, dGTP and dTTP, 2.0 U of Taq DNA polymerase (Perkin Elmer, NJ USA), and LRP-specific oligonucleotide primers (50 pmol of the sense primer 5'-GCAGTGTCTACCGCTTGGAA-3', corresponding to nt 2754-2773, and 50 pmol of the antisense primer 5'-TGGACTCATCTT CACTGTTC-3', complementary to nt 3229-3248) (26). Each cycle consisted of denaturation at 94˚C for 60 s, primer annealing at 55˚C for 60 s, extension at 72˚C for 60 s, and a final extension at 72˚C for 7 min in a RoboCycler 96 Temperature Cycler (Stratagene, La Jolla, CA USA) (27). The efficiency of cDNA synthesis from each tissue sample was estimated by PCR with GAPDH-specific primers. GADPH cDNA was amplified with primers corresponding to nt 27-46 (5'-ACGGATTTGGTCGTATTGGG-3') and complementary to nt 238-257 (5'-TGATTTTGGAGGGATCTCGC-3') (28) under the same conditions as used for LRP.

Samples of each LRP (10 μl) and GAPDH (10 μl) PCR product were electrophoresed in 1.5% agarose gel and photographed as ethidium bromide fluorescent bands. The PCR procedure was performed at least three times for each sample. After amplification, 40 μl of the PCR products of LRP were electrophoresed on a 3% agarose gel. The amplified bands were cut out, eluted and subjected to direct sequencing to confirm the identity of LRP transcript by an automated DNA sequencer (ABI377).

Preparation of photosensitizer (Photofrin). Photofrin was purchased from QTL Photo Therapeutics Inc., Canada) dissolved in 5% dextrose in water at a concentration of 7.5 mg/ml and stored at 20˚C until use. The cells were incubated for 60 min in media containing Photofrin (75 μg/ml final concentration) at 4˚C in a dark room and then sonicated as described elsewhere (16). For the control experiment, the cells were incubated in media adding same amount of 5% dextrose as the Photofrin solution.

Ultrasound source and experimental protocols. The ultrasound apparatus (E-KON, USA) with a resonance frequency of 0.95 MHz was used in all the sonication experiments (16). One milliliter of the cell suspensions was placed in a Pyrex test tube (12 mm diameter, 100 mm in length). An ultrasound emitting transducer (6×6×0.62 mm) was inserted directly into the cell suspension (1×10^6 cells/ml). Cells were sonicated with continuous wave ultrasound with or without presence of photosensitizer Photofrin (75 μg/ml) at an intensity of 0.3 W/cm^2 for duration of 5, 15, or 30 s. Temperature changes of cell suspension immediately before and after treatment by ultrasound were measured by a needle thermometer (Tele-Thermometer; Yellow Springs Instrument Company Inc., OH, USA). All experiments were repeated three times for each condition.

Sonicated cells with or without Photofrin (75 μg/ml) at an intensity of 0.3 W/cm^2 for 30 s were observed with a scanning electron microscope (S450; Hitachi, Ltd., Tokyo, Japan) operating at 10 kV (16).

Pretreatment by RAP before sonication with Photofrin. The cells were preincubated with RAP (100 nM and 10 nM) (29) at 4˚C for 60 min and then incubated with Photofrin (75 μg/ml final concentration) at 4˚C for 60 min in a dark room, before being sonicated at an intensity of 0.3 W/cm^2. Aliquots of cell suspension before sonication were collected by centrifugation and then washed twice with phosphate-buffered saline. Approximately 5 million intact cells were taken from each sample, suspended in 2 ml of 0.1 N NaOH and mechanically lysed using a sonicator (Polytron PT1200; KinematicaAG, Littau, Switzerland). Intracellular Photofrin

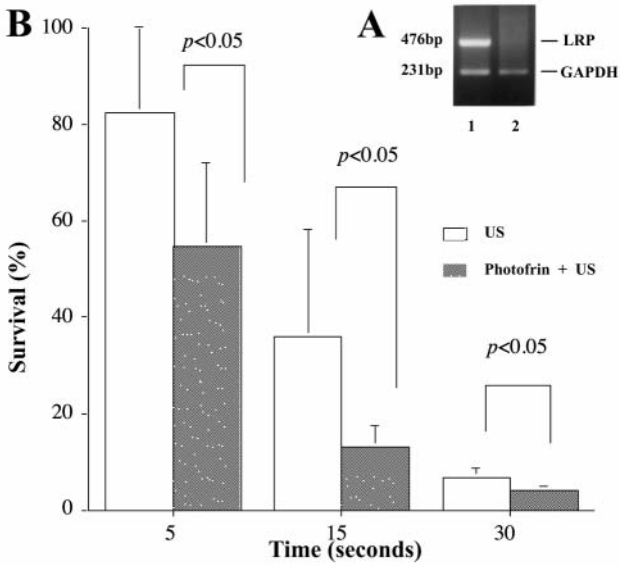

Figure 1. *LRP/α2MR expression in glioma cell lines and survival for U251MG cells after ultrasound sonication. A, Total RNA was isolated from U251MG (Lane 1) and U105MG (Lane 2), reverse transcribed and PCR amplified for LRP/α2MR. B, Enhancement of ultrasound (US)-induced cell death by Photofrin in U251MG cells. U251MG cells were incubated with or without Photofrin at 75 mg/ml for 60 min prior to US exposure. Average of three experiments; bar, SE.*

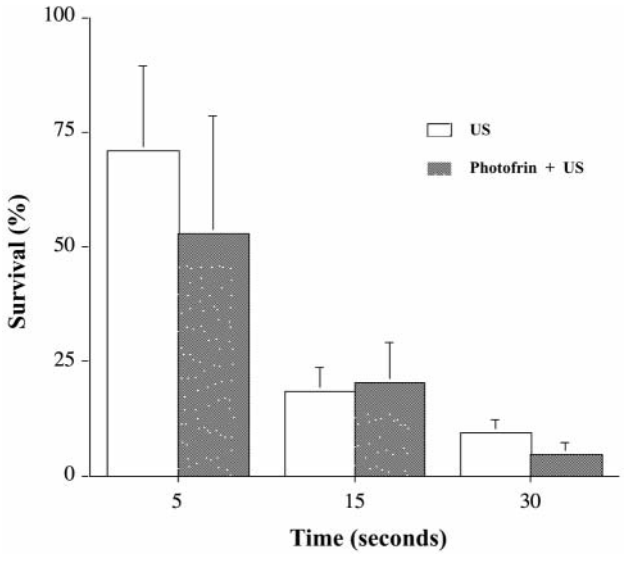

Figure 2. *Survival of U105MG cells after ultrasound sonication. Enhancement of ultrasound (US)-induced cell death by Photofrin in U105MG. U105MG cells were incubated with or without Photofrin at 75 mg/ml for 60 min prior to US exposure. Average of three experiments; bar, SE.*

concentration was determined by absorption spectroscopy in the supernatant of each lysate (25). Reference samples contained extraction solution from an equal cell number which had not been incubated with Photofrin. A standard curve was obtained by adding known amounts of Photofrin to appropriate cell numbers prior to performing identical extraction procedures using absorption ratio measurements at 390 and 470 nm. A part of the cell suspension was used for protein determination *via* the Lowry assay. Photofrin levels were calculated on the basis of μg Photofrin/mg protein.

Measurement of cell viability. Immediately after treatment, cell viability was determined with the trypan blue exclusion test by adding 15 μl trypan blue solution to 15 μl cell suspension, and counting unstained cells using a Burker Turk hemocytometer to estimate the survival immediately after sonication. The number of surviving cells before exposure to ultrasound was set as 100% .

Statistics. Student's *t*-test was used to compare the survival between the two groups. One way analysis of variance (ANOVA) was used to compare the Photofrin uptake in glioma cell lines with or without RAP pretreatment. All of the data were analyzed using a contemporary statistical package (SPSS 12.0J; Chicago, IL, USA). $P<0.05$ was taken as the level of significance for all tests.

Results

Enhancement of ultrasound cell killing with Photofrin in LRP/α2-MR-expressing glioma cells. To determine the appropriate ultrasound intensity for sonication in human glioma cells, we examined the survival after sonication at ultrasound intensity varied from 0.15, 0.2 to 0.3 W/cm^2 for 30s in U251MG. Survival rates decreased from 86.8±6.10% , 35.6±34.1% to 9.8±2.9% respectively as ultrasound intensity increased. Thus, on ultrasound intensity of 0.3 W/cm^2 was selected for further experiments.

The expressions of LRP/α2-MR mRNA were evaluated by RT-PCR of cDNA prepared from the U251MG and U105MG human glioma cell lines. Oligonucleotide primers in this study were used to amplify 476-base cDNA for LRP/α2-MR. LRP/α2-MR was present in U251MG (Figure 1A, *Lane 1*), while LRP/α2MR was undetectable in U105MG (Figure 1A, *Lane 2*). No gene products of interest were amplified using PCR when total RNA extracts from these cell lines were incubated in reverse transcriptase reactions without reverse transcriptase (data not shown). Direct sequencing analyses of RT-PCR products showed that LRP/α2MR transcript was identical.

Cells were sonicated with continuous wave ultrasound with or without presence of photosensitizer Photofrin (75 μg/ml) at an intensity of 0.3 W/cm^2 for duration of 5, 15, or 30 s. Ultrasound alone induced instant cell immediately after sonication in both U251MG (Figure 1 B) and U105MG (Figure 2) cells. Survival decreased as sonication was prolonged, 82.3±18.1% , 36.0±22.4% and 6.7±2.2% in U251MG cells for 5, 15 and 30 s respectively, and 70.7±18.8% , 18.3±5.6% and 9.5±2.8% in U105MG cells for 5, 15 and 30 s respectively. In U251MG, which expressed

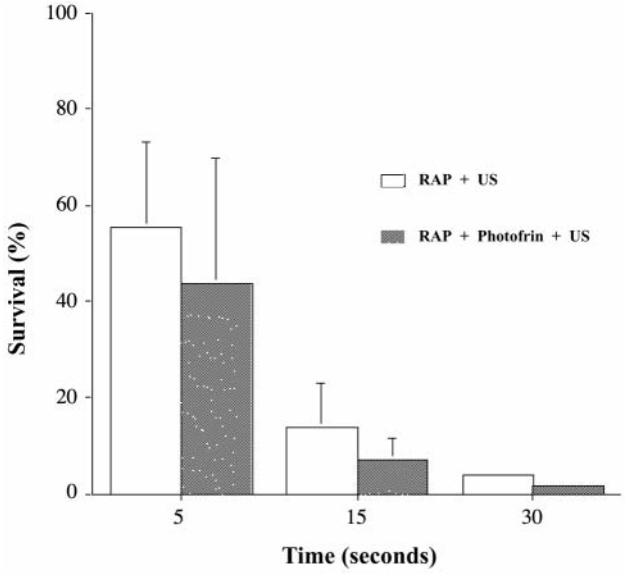

Figure 3. *Inhibition of Photofrin-enhanced ultrasound cell killing by RAP pretreatment in U251MG cells. The cells were preincubated with 100 nM RAP prior US exposure with or without Photofrin at 75 μg/ml for 60 min pretreatment. The viability of the cells was measured using the Trypan blue dye exclusion test after sonication.*

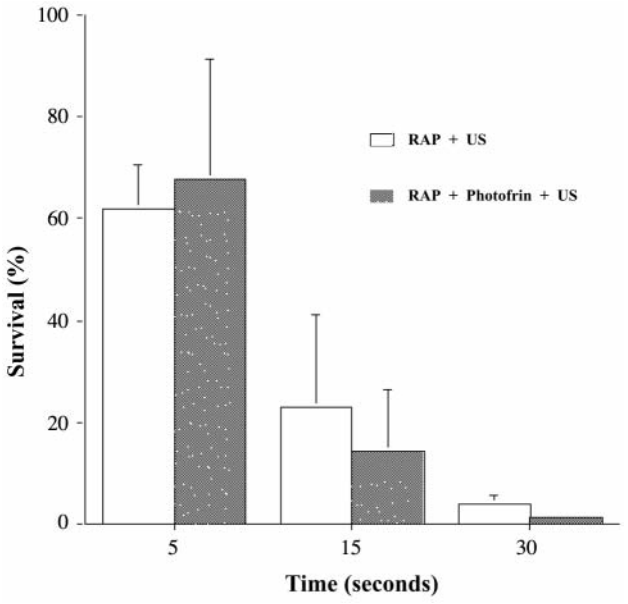

Figure 4. *Inhibition of Photofrin-enhanced ultrasound cell killing by RAP pretreatment in U105MG cells. The cells were preincubated with 100 nM RAP prior to US exposure with or without Photofrin at 75 mg/ml for 60 min pretreatment. The viability of the cells was measured using the Trypan blue dye exclusion test after sonication.*

LRP/α2MR, significantly higher cell killing was observed following Photofrin pretreatment (Figure 1B), 52.7±17.5% , 13.0±4.6% , and 3.9±0.9% for 5, 15 and 30 s respectively (p<0.05) while enhancement of ultrasound-induced cell death by Photofrin was not achieved in U105MG cells (Figure 2; survival of 52.9±25.4% , 20.1±9.1% and 4.8±2.4% for 5, 15 and 30 s respectively). Photofrin alone at the concentration used showed no cytotoxicity (not shown). Temperature changes before and immediately after ultrasound exposure at all intensities and durations were less than 0.5˚C.

Inhibition of Photofrin intracellular uptake and enhancement of ultrasound cell killing by RAP pretreatment. Cells were pretreated with 100 nM RAP and incubated with and without Photofrin (75 μg/ml) before being sonicated with continuous wave ultrasound at an intensity of 0.3 W/cm^2 for a duration of 5, 15, or 30 s. Survival decreased as sonication was prolonged, being 55.4±17.9% , 13.8±9.2% and 3.9±0.7% in U251MG cells with 5, 15 and 30 s sonication respectively, with RAP pretreatment (Figure 3). In ultrasound with RAP and Photofrin pretreatment, survivals were 43.6±26.1% , 6.9±4.7% and 1.8±0.7% in U251MG cells sonicated with 5, 15 and 30 s respectively (Figure 3). There was no significant difference between ultrasound plus RAP group and ultrasound plus RAP and Photofrin group in the cell killing effect. This showed that the enhancement of cell killing with Photofrin was diminished by RAP pretreatment.

Survival also decreased as sonication was prolonged being 61.9±8.6% , 22.8±18.2% , and 3.6±1.7% in U105MG cells sonicated for 5, 15 and 30 s respectively, with RAP pretreatment (Figure 4). In ultrasound with RAP and Photofrin pretreatment survivals were 67.4±23.7% , 14.2±12.3% and 1.0±0.4% in U105MG cells sonicated for 5, 15 and 30 s respectively (Figure 4). There was no significant difference between ultrasound plus RAP group and ultrasound plus RAP and Photofrin group in the cell killing effect. There was no inhibition of the cell killing effect when cells were pretreated with 10 nM RAP in U251MG cells. RAP alone at 100 nM showed no cytotoxicity (not shown).

Cellular Photofrin uptake following RAP incubation in the photosensitizer experiments is shown in Table I. U251MG cells accumulated 2.43±0.25 Photofrin μg/mg protein, which was significantly reduced with RAP pretreatment (1.38±0.22 Photofrin μg/mg protein) (p<0.05). U105MG cells accumulated 1.31±0.16 Photofrin μg/mg protein, which was less than in U251MG cells. Photofrin uptake was not altered by RAP pretreatment in U105MG cells.

Induction of cell membrane porosity by ultrasound. Intact, U105MG (Figure 5A) and U251MG (Figure 5B) glioma cells were covered with microvilli on the cell surface. Exposure to ultrasound at an intensity of 0.3 W/cm^2 for 30 s in the absence of Photofrin caused minor disruptions of the surface in U105MG (Figure 5C) and U251MG (Figure 5D)

Table I. *Inhibition of Photofrin intracellular uptake by RAP pretreatment in human glioma cells.*

Cell type	Photofrin (75 μg/ml)	RAP (100 nM)	Photofrin uptake (μg/mg protein)
U251	+	–	2.43±0.25[a]
	+	+	1.38±0.22[b]
U105	+	–	1.31±0.16[a]
	+	+	1.23±0.19[a]

[a]Mean±SE, n=3; [b]$p<0.05$.

cells. The microvilli on the cell surface disappeared and several flap-like wrinkles were seen in both U105MG and U251MG cells. U251MG cells exposed to ultrasound in the presence of Photofrin had multiple surface pores and dimple-like craters (Figure 5F). In contrast, U105MG cells exposed to identical ultrasound conditions with Photofrin showed none of these features (Figure 5E). This remarkable difference in cell surface morphology suggests a completely different ultrasound cell-damaging phenomenon induced in the presence of Photofrin. No structural changes as compared with intact cells were observed in cells with Photofrin alone without ultrasound irradiation (not shown).

Discussion

In this study, we found that a cell killing effect could be achieved with low-level ultrasound in human glioma cells. Two different human glioma cells, U251MG and U105MG, were examined in this study and both showed sensitivity to low-level ultrasound. The survival of both of these cell lines decreased markedly as ultrasound duration was prolonged. However, there was a significant difference in response to the photosensitizer Photofrin. Enhancement of ultrasound-induced cell death by Photofrin was only observed in the U251MG cell line, which expressed LRP/α2MR. The concentration of intracellular Photofrin was higher in U251MG than that in U105MG cells, which did not express LRP/α2MR. The enhancement of ultrasound-induced cell killing by Photofrin was abolished by pretreatment with RAP, which specifically binds to LRP/α2MR. These results show that enhancement of ultrasound-induced cell killing by Photofrin depends on the extent of intracellular Photofrin uptake.

Intracellular Photofrin uptake was inhibited by RAP treatment in the U251MG cell line, which expressed LRP/α2MR. Intracellular uptake of Photofrin in U251MG cells was not completely inhibited by RAP pretreatment, and in U105MG cells, Photofrin uptake was not changed at all which suggested that intracellular uptake of Photofrin was only partially mediated by LRP/α2MR. Other than a simple diffusion mechanism, another mechanism, such as receptor-mediated uptake, was reported in tumor cells because Photofrin is preferentially taken up and retained by tumor cells (11, 22). LRP/α2MR is a member of the LDL receptor family and binds with high affinity, endocytosing several structurally and functionally distinct ligands (30, 31). In addition to its role in lipoprotein metabolism (32), many other ligands can be internalized upon binding to LRP/α2MR, including *Pseudomonas* endotoxin A (33), a 39-kDa protein which is also called receptor-associated protein (RAP) (34), α2-macroglobulin-protease complexes (α2M) and plasminogen activator-inhibitor complexes (35, 36). In human tissues, LRP/α2MR is mainly expressed in the placenta and liver (37). It has been reported that LRP/α2MR expression decreases with aggressiveness in several human tumor cell lines, lung carcinoma, osteosarcoma and cervical epithelium, compared with nontumor cell lines (38, 39). However, Bu *et al*. demonstrated the expression and endocytic function of LRP/α2MR in the glioblastoma cell line U87 (29). We have previously reported that malignant gliomas, especially glioblastoma, expressed abundant LRP/α2MR (27). LRP/α2MR was almost undetectable in normal glial cells and endothelial cells of normal brain tissues around the tumor. It has been reported that LDL receptor plays an important role in the transport and delivery of hydrophobic photosensitizers including Photofrin (23, 24). Luna *et al*. also demonstrated that a lack of LRP/α2MR expression caused reduced intracellular uptake of Photofrin PDT-resistant in tumor cells (25). The photosensitizer hematoporphyrin derivative (HpD) has been shown to be selectively localized into all grades of gliomas (40). 5-Aminolevulinic acid (5-ALA) is specifically taken up by cancer cells and converted to photosensitizing concentrations of protoporphyrin IX (PpIX) (41). Together with these previous reports, our results suggest that LRP/α2MR, the unique multifunctional receptor protein, mediates intracellular uptake of Photofrin in certain glioblastoma cells, which enables enhancement of low-intensity ultrasound-induced tumor cell killing.

Ultrasound energy has been extensively investigated and used over the past three decades in a wide range of clinical procedures (42). Sonication with high-intensity focused ultrasound is an effective local cancer treatment that induces cytotoxicity through thermal effects and nonthermal cavitation which generates intracellular reactive oxygen species (43, 44). Nonthermal sonolytic effectiveness of a given low level ultrasound exposure has been correlated with the generation of acoustic cavitation (20, 45). Ultrasonically-induced cavitation, defined as generation and oscillation of gas bubbles, may cause irreversible cell damage and modify the membrane structure and functional properties of the cells to induce cell killing by cell lysis, necrosis or apoptosis (17, 44, 46, 47). Nonthermal effects of ultrasound may have the greatest potential for therapeutic applications for deep-seated or invasive tumors because ultrasound can penetrate intervening tissues (21). Ablation

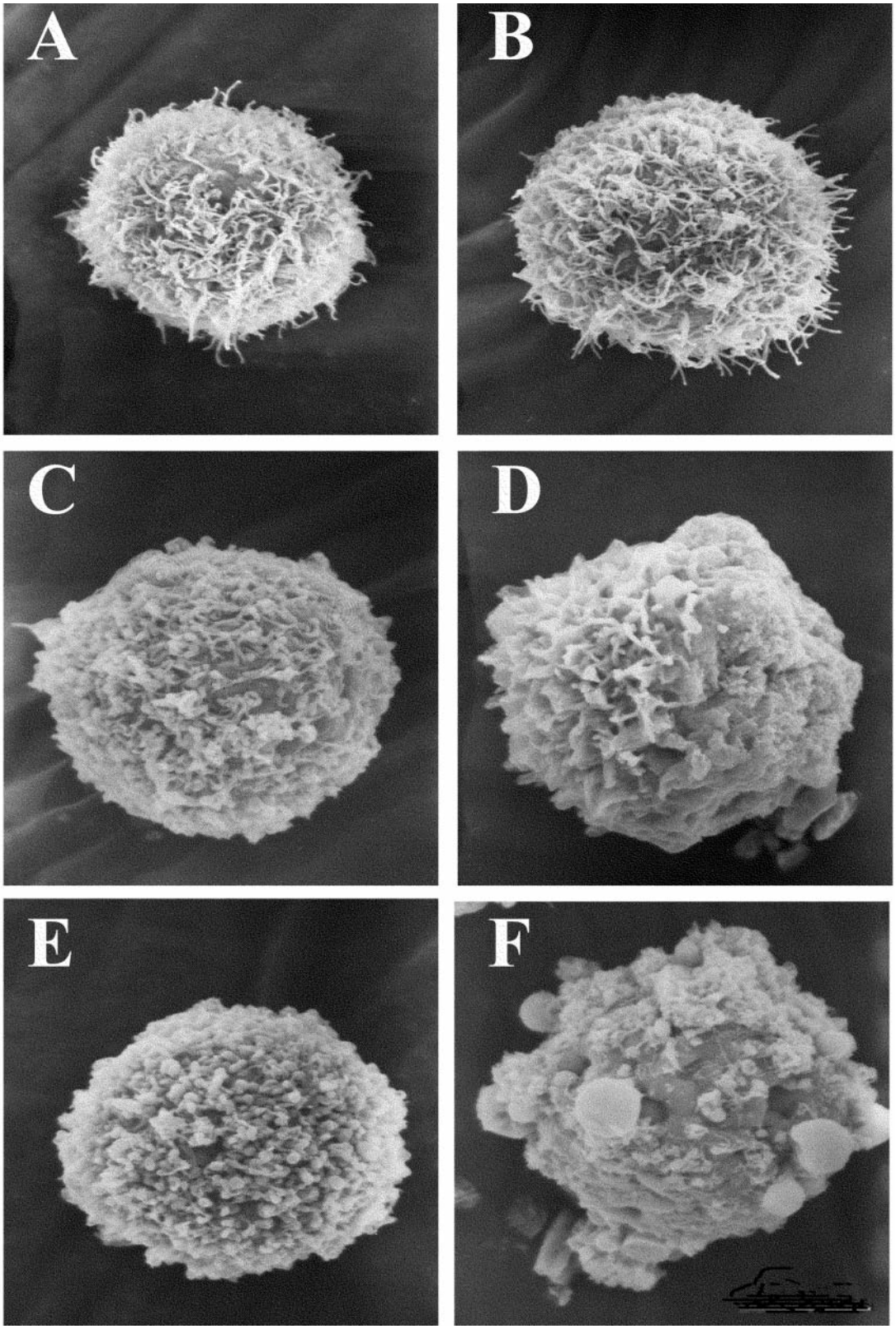

Figure 5. *Induction of cell-membrane porosity by ultrasound. Sonicated cells with or without Photofrin (75 μg/ml) at an intensity of 0.3 W/cm^2 for 30 s were observed with a scanning electron microscope operating at 10 kV. A, Untreated intact U105 MG cells; B, U251 MG cells; C, U105MG cells and D, U251MG cells irradiated with US alone; E, U105MG cells and F, U251MG cells exposed to US in the presence of 75 μg/ml Photofrin.*

of adult T-cell leukemia cells and lysis of HL-60 cells by low level ultrasound was enhanced in the presence of a photosensitizing chemicals, indicating that the photosensitizer potentiates the cytotoxicity of ultrasound (15, 16). Photosensitizers become activated to be very cytotoxic, which leads to cell membrane damage. These effects of activated photosensitizer are similar to those of photodynamic therapy (8, 48, 49).

The net effect of all this cellular damage is necrosis and apoptosis. Selective intracellular uptake of photosensitizer by receptor-mediated mechanisms in tumor cells can enable low-intensity ultrasound to achieve selective antitumor effects against intracranial gliomas sparing normal brain tissue damage around the tumor. Human malignant glial tumors constitute the majority of primary intracranial tumors and are often characterized by rapid growth and aggressive invasion into surrounding normal brain tissue (50). Malignant glioma cell migration and invasion clearly involve a complex interplay of multiple proteolytic enzymes and their inhibitors (51). This infiltrative nature of malignant glioma causes the failure of curative treatment, with most cases recurring at the site of the original tumor (52). These infiltrative tumor cells in surrounding normal brain tissues are good targets for ultrasound therapy that combines low-intensity ultrasound and photosensitizer. This treatment can be applied to intracranial gliomas *via* burr hole surgery and intraoperative irradiation to eliminate the invasive tumor cells surrounding normal brain tissue. Intraventricular tumor including intraventricular disseminated tumor, glioblastoma, and germ-cell tumors could also be good candidates for this ultrasound irradiation using endoscopy (53). A catheter with a low-intensity ultrasound probe is now in clinical trials.

We are now working on an experiment on low-intensity ultrasound with HpD and 5-ALA for the treatment of experimental intracranial glioma in rat to confirm if a selective antitumor effect can be achieved *in vivo*. It also remains to be elucidated whether low-intensity ultrasound exposure with Photofrin improves the long-term survival of rats with experimental glioma.

To our knowlege, this is the first report to demonstrate the usefulness of low-intensity ultrasound combined with photosensitizer for the cellular killing of malignant gliomas cells. This could shed new light on how to prevent local tumor recurrence of malignant glioma, which might improve the treatment outcome in glioblastoma.

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