Abstract. Aim: To determine whether the use of high-intensity focused ultrasound (HIFU) ablation combined with cisplatin, compared with HIFU ablation, and cisplatin alone, facilitates tumor necrosis and reduces tumor volume in an animal model. Materials and Methods: The cervical cancer cell line SiHa was cultured and injected subcutaneously into female BALB/c nude mice. Animals that developed tumors were randomized to four groups: treatment with HIFU ablation and cisplatin (i.p. injection, 100 mg/mouse twice per week); cisplatin treatment alone; HIFU ablation alone; and control. The dimensions of the tumor were measured transcutaneously using a vernier caliper. After mice were sacrificed, tumor excised, and triphenyl tetrazolium chloride (TTC) staining was carried out to investigate the area of coagulation necrosis in the tumors. Results: Out of the 32 animals that developed tumors, 25 had tumors able to be measured through subcutaneous palpation in the pretreatment period (control, n=7; HIFU ablation, n=7; cisplatin, n=6; combination of HIFU ablation and cisplatin, n=5). In the group receiving HIFU ablation with cisplatin, tumor volume decreased significantly when compared with the other groups. TTC staining showed that necrosis was induced in the central zone of the tumors that were ablated by HIFU, and chemotherapy enhanced the effect of HIFU ablation in the combination therapy group. Conclusion: HIFU ablation combined with cisplatin facilitates reductions in tumor volume and increases in tumor necrosis and could be useful as another option in treating patients with cervical cancer.

Cervical cancer, which is the second most common type of cancer among women in developing countries (1) and the leading cause of cancer death in women on a global scale (2), is a significant healthcare problem. With programs promoting screening for cervical cancer, it is being diagnosed at earlier stages, leading to better survival rates (3). The age distribution of cervical cancer is bimodal, with peaks at 35 to 39 years and 60 to 64 years of age (3). More than 25% of women with cervical cancer are under 40 years of age and the age of nulliparous women with this disease has increased (4). Young patients who are diagnosed with early-stage cervical cancer have high cure rates (5). However, radical surgery and radiotherapy do not spare fertility and both methods can lead to psychosexual dysfunction and decreased quality of life (6). Furthermore, infertility was found to increase the frequency of depression, stress, and sexual dysfunction (7). Conservative surgery such as fertility-sparing surgery for some patients has emerged recently to overcome the limitations of the current standard management (8), but experience with this therapeutic modality is limited by the uncertainty of long-term outcomes (9).

On the other hand, recurrent or metastatic cervical cancers are not considered curable with chemotherapy and in these cases palliative radiation therapy is used to relieve symptoms of pain or bleeding associated with the disease. However, special care should be given to previously irradiated sites because additional radiation therapy may be associated with unacceptable morbidity and, unfortunately, symptomatic recurrent disease within previously irradiated fields may not respond well to palliative chemotherapy (5). Therefore, new treatment options for cervical cancer are needed.
High-intensity focused ultrasound (HIFU) ablation therapy is an emerging therapeutic modality using high-intensity focused ultrasound to destroy tumors through thermal ablation. This non-invasive technique has been demonstrated to be of particular value in curative treatment and the extension of life expectancy for patients with many types of solid cancer, such as liver, pancreatic, breast, bone, kidney, and prostatic malignancies (10, 11). However, the role of HIFU ablation, especially combined with concurrent systemic chemotherapy, on cervical cancer is not known. Herein, we present the results of a study investigating whether concurrent chemotherapy and HIFU ablation have a therapeutic role for cervical carcinoma in an athymic nude mouse model.

Materials and Methods

Cell culture conditions. The cervical cancer cell line SiHa was cultured in MEM (Gibco, NY, USA) supplemented with 10% fetal bovine serum and 1% antibiotics. For in vivo injections, cells were trypsinized and centrifuged at 1,200 rcf for 3 min at 4°C, washed twice with phosphatebuffered salline (PBS) (Wegene, Daegu, Republic of Korea), and reconstituted in serum-free Hank’s balanced salt solution (HBSS) (Gibco, Carlsbad, CA, USA). Only single-cell suspensions with >95% viability, as determined by trypan blue exclusion, were used for in vivo injections. All experiments were carried out using cells grown to 60% to 80% confluence, and the cell line was routinely tested to confirm the absence of mycoplasma.

Animal care and tumor implantation. Female BALB/c nude mice were purchased from Orient Bio (Sungnam, Korea). This study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Samsung Biomedical Research Institute (SBRI). SBRI is a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International, protocol No. H-A9-003) and abides by the Institute of Laboratory Animal Resources (ILAR) guide (12).

To produce tumors, SiHa cells (1.0x10^6/0.2 ml HBSS) were injected subcutaneously in the thigh area to minimize the effects from breathing movement during HIFU ablation. The mice used in these experiments were six weeks old. Mice (n=8 per group) were monitored daily for tumor development and post-treatment complications.

Treatment. A commercially available animal experiment system (Therapy Imaging Probe System, Philips Research, Briarcliff Manor, NY, USA) was used to generate the HIFU. Both the natural focus and the diameter of this annular array transducer are 80 mm, and the size of the focal zone is 1 mm x1 mm x6 mm at ~6 dB. Each tumor-bearing mouse was anesthetized via ventilation with the use of isoflurane and fixed on the acoustic absorber and the custom-made treatment bed. Acoustic coupling between the transducer and the target tumor was made with degassed water (dissolved oxygen level <3 ppm) with a temperature maintained at 36.5°C by a thermostat (Figure 1).

HIFU was applied to the tumor with the following parameters: i) total acoustic power, 30 W; ii) acoustic frequency, 1.0 MHz; iii) sonication for 10 s and cooling for 10 s (i.e. duty cycle, 50%) per sonication spot; and iv) spacing between each sonication spot, 1 mm. HIFU sonication covered the central part of the tumor in a 3 mm x3 mm raster grid pattern (i.e. nine spots per tumor).

The long and short dimensions of the tumor were measured transcutaneously using a vernier caliper. Because nude mice have thin skin, no hair, and little subcutaneous fat, no correction for skin thickness was made. Tumor volume was calculated based on the assumption that each tumor was a regular ellipsoid [V=4/3πabc], where a, b, and c are the distances from the center to the edge on each axis]. Animals that developed tumors were randomized to one of four treatment groups: HIFU ablation and cisplatin (i.p. injection, 100 μg/mouse twice a week); HIFU ablation alone; cisplatin (Sigma, St. Louis, MO, USA) treatment alone, and control. Treatment was administered when the average tumor volume was approximately 0.1 cm^3 (approximately 0.6 cm in diameter). In the control group, tumors were allowed to grow to a maximum of 10% of the animal’s total body weight before the animals were euthanized.

Triphenyl tetrazolium chloride (TTC) staining. Removed tumors were immersed for 30 min in a freshly made 1% TTC solution at 37°C. TTC staining results in viable tissue staining a ‘brick-red’ color as the tetrazolium salts react with the dehydrogenases in the cells, and the non-viable tissue stains a pale-white since those cells lack the enzymes with which the TTC reacts.

Statistical methods. Comparisons of means or medians were performed using one-way ANOVA as a parametric test or the Kruskal-Wallis test as a non-parametric test. Statistical analyses were performed using SPSS software (version 12.0; SPSS, Chicago, IL, USA). A p-value ≤0.05 was considered statistically significant and all p-values were two-sided.

Results

Out of the 32 animals that developed tumors, 25 had tumors able to be measured through subcutaneous palpation (approximately 0.6 cm in diameter) in the pretreatment period (control, n=7; HIFU, n=7; cisplatin, n=6; combination of HIFU and cisplatin, n=5; Table I). We did not find skin burns at the treatment sites in any animal.

All tumors had similar growth rates and volumes before treatment. Figure 2 shows a graph of the relative average tumor volumes for the four groups as a function of time, with
data normalized so that all treatments were applied at day 0. In the group which underwent HIFU ablation with cisplatin, tumor volume decreased significantly when compared with volumes of the other groups including control, HIFU ablation alone, and cisplatin alone, in which tumors continued to grow during the follow-up period.

TTC staining revealed that red compound was dominant in the control group and cisplatin-alone treatment group, while white compound was prominent in the HIFU ablation alone group and was even more prominent in the combination group, which underwent HIFU ablation and

Figure 1. Experimental set-up for high-intensity focused ultrasound (HIFU) ablation. A tumor-bearing mouse under ventilation anesthesia was fixed on the acoustic absorber and the custom-made frame. The HIFU transducer was obliquely placed with its focus in the tumor. Acoustic coupling between the HIFU transducer and the tumor was made with degassed water for which oxygenation was minimized by a layer of small floating plastic balls.

Figure 2. A plot of average tumor volume (% of initial size) as a function of time in each treatment group. The error bars indicate two standard errors of the mean. Treatment, if applied, was at day 0. HIFU: high-intensity focused ultrasound.

Figure 3. Triphenyl tetrazolium chloride staining of tumor from: Live cells, red; dead cells, white. HIFU: high-intensity focused ultrasound.
chemotherapy. This finding indicates that necrosis was induced in the central zone of the tumor, which was ablated by HIFU, and that chemotherapy enhanced the effect of HIFU ablation in the combination group (Figure 3).

**Discussion**

In this study, we found that HIFU ablation with concurrent systemic chemotherapy using cisplatin significantly reduced tumor volumes in a xenograft cervical cancer model. Furthermore, tumors treated by HIFU ablation contained profound necrotic tissue within the tumor mass.

Since 1942 when evidence was found that focused ultrasound ablation could be achieved as a result of controlling local heating phenomena (13), the HIFU technique has been improved and several clinical studies were performed for various malignant diseases (14-16), where the safety and feasibility of this technique in clinical application was demonstrated. More recently, there have been attempts to investigate the therapeutic effects of HIFU in combination with other treatments for advanced solid tumors, such as HIFU combined with immunotherapy for urological malignancies (17), or HIFU with tumor embolization for hepatocellular carcinoma (18). In addition, there have been efforts to use nanoparticles to boost the therapeutic effect of HIFU (19). One experimental study on HIFU ablation combined with chemotherapeutics demonstrated a potential synergistic inhibitory effect for HIFU plus paclitaxel and estramustine phosphate in a rat model of prostate cancer (20), yielding results similar to those of our study. In addition, ThermoDox (Celson Corporation, Columbia, MD, USA), which is heat-activated liposomal encapsulation of doxorubicin, is currently undergoing a clinical trial for treatment of hepatic and breast carcinomas (21).

The potential utility of HIFU in cervical cancer is expected to be high. HIFU ablation may have a role not only in early cervical cancer as mentioned in the introduction but also in the management of advanced or recurrent cervical cancer. For example, there was a report that concurrent gemcitabine and HIFU ablation was a tolerated treatment modality with promising activity in patients with previously untreated locally advanced pancreatic cancer (22). Furthermore, HIFU therapy should be applicable to patients who showed resistance to radiotherapy or patients receiving palliative care to reduce tumor-related symptoms, such as pain (14).

To see the therapeutic effect of chemotherapy on tumors in combination with HIFU ablation, our study was designed to deliver a sublethal dose of HIFU focusing only on the small central area (3×3×6 mm) within the tumor, not the whole area of the tumor, which could have prevented detection of the tumoricidal effect of HIFU ablation alone. In an *in vitro* study, HIFU-induced gene activation was observed in sublethally injured cells (23), and this change in gene expression may be associated with tumor re-growth after HIFU therapy with a sublethal dose. However, we also found dead tissue in the central zones of tumors in the HIFU ablation-alone group and these necrotic areas were larger and extended to the peripheral zone of tumors in the HIFU ablation and chemotherapy combination group, suggesting a synergistic effect between HIFU ablation and chemotherapy.

Concurrent HIFU ablation and chemotherapy has been reported to induce apoptosis and inhibit tumor growth to a greater extent than is achieved with each treatment alone (24-26). Enhancement of drug extravasation and increased sensitization of the cancer cells to chemotherapeutic agents can be achieved with local hyperthermia, which can increase drug delivery by inducing an increase in blood flow to the target tissues, or mechanical augmentation, such as acoustic cavitation, radiation force, shear stress, and acoustic streaming/microstreaming, which can induce structural and molecular changes (12, 24, 27).

An HIFU system designed for patients with gynecological benign tumors is now available (28). However, to apply the HIFU treatment to patients with early-stage cervical cancer, a new device designed to be used transvaginally must be developed because most of the clinically available devices are for an extracorporeal system, which is for general purposes, or a transrectal system, which is only for patients with prostate cancer. In addition, further studies to investigate the role of HIFU therapy in palliative treatment combined with chemotherapy for patients with recurrent or metastatic cervical cancer are warranted using the existing devices.

In conclusion, HIFU ablation combined with concurrent systemic cisplatin therapy facilitates a decrease in tumor volume, probably due to an increased area of tumor necrosis, and may be useful for the treatment of patients with cervical cancer.

**Conflicts of Interest**

The Authors declare that there are no conflicts of interest.

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**References**


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5289