Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 335 (2005) 124–131
BBRC
www.elsevier.com/locate/ybbrc

Release of endogenous danger signals from HIFU-treated tumor cells and their stimulatory effects on APCs

Zhenlin Hu [a], Xiao Yi Yang [b], Yunbo Liu [a], Michael A. Morse [b], H. Kim Lyerly [b], Timothy M. Clay [b], Pei Zhong [a,*]

[a] *Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC 27708, USA*
[b] *Program in Molecular Therapeutics, Departments of Surgery, Medicine, Pathology, and Immunology, and Duke Comprehensive Cancer Center, Duke University, Durham, NC 27708, USA*

Received 11 July 2005
Available online 26 July 2005

Abstract

The effects of high-intensity focused ultrasound (HIFU) on the release of endogenous danger signals from tumor cells and subsequent activation of antigen-presenting cells (APCs) were evaluated in vitro. MC-38 mouse tumor cells were treated using a 1.1 MHz HIFU transducer under two different protocols ($P^- = 6.7$ MPa, 30% duty cycle, 5 s vs. $P^- = 10.7$ MPa, 3% duty cycle, 30 s) to produce either thermal necrosis or mechanical lysis of the tumor cells. Here, we report that HIFU treatment can cause the release of endogenous danger signals (ATP and hsp60) and exposure of dendritic cells (DCs) and macrophages to the supernatants of HIFU-treated tumor cells leads to an increased expression of co-stimulatory molecules (CD80 and CD86) with enhanced secretion of IL-12 by the DCs and elevated secretion of TNF-α by the macrophages. The potency in APC activation produced by mechanical lysis is much stronger than thermal necrosis of the tumor cells. These findings suggest that optimization of treatment strategy may help to enhance HIFU-elicited anti-tumor immunity.
© 2005 Elsevier Inc. All rights reserved.

Keywords: High-intensity focused ultrasound; Endogenous danger signals; Antigen-presenting cells; Anti-tumor immunity

In recent years, high-intensity focused ultrasound (HIFU) has emerged as a promising non-invasive treatment modality for a variety of cancers, including breast, prostate, kidney, liver, bone, uterus, and pancreatic cancers [1]. The fundamental principle of HIFU is to produce coagulative tissue necrosis in a well-defined region by converting focused acoustic energy into heat. The beam size of a typical HIFU transducer is of the order of 10×1 mm (longitudinal × transverse direction). Therefore, treatment of a tumor is usually accomplished by progressive scanning of the whole tumor volume line-by-line and layer-by-layer. Extensive animal studies have been carried out [2–4] and several clinical trials [5–7] are currently underway to establish the appropriate treatment protocols for HIFU applications. In addition to its inherent advantage of non-invasiveness, HIFU treatment is also well-tolerated by patients and thus can be administered, repetitively. Despite these advantages, several drawbacks still exist in the current form of HIFU therapy for cancer. These include: (1) incomplete tissue necrosis especially in large tumors, presumably due to inhomogeneities in tissue properties and heat conduction [8], (2) inability to kill metastatic cancer cells outside the primary tumor site, and (3) long treatment duration (up to several hours). Although technical improvement in HIFU technology such as the use of phase-array systems for electronic scanning may eventually reduce the treatment time, the first two drawbacks have to be amended before HIFU can be truly established as a primary treatment modality for cancers.

* Corresponding author. Fax: +1 919 660 8963.
E-mail address: pzhong@duke.edu (P. Zhong).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.07.071

Several clinical studies have suggested that, in addition to its thermal ablation capability, HIFU may also modulate the immune response of the patients. For example, following HIFU treatment, a marked increase in CD3+ and CD4+ subsets, and the CD4+/CD8+ ratio in peripheral blood of cancer patients has been observed [6,9,10]. In addition, Yang et al. [11] reported that HIFU treatment of subcutaneous murine C1300 neuroblastoma could cause a significant reduction in tumor growth upon subsequent tumor challenge. Although these preliminary data indicate that HIFU may enhance host anti-tumor immunity, this speculation has not been confirmed rigorously using well-established animal models and the underlying mechanisms are largely unknown. Despite this, the potential for HIFU-elicited anti-tumor immunity warrants serious consideration. This is because if HIFU can indeed elicit anti-tumor immunity and such an effect can be enhanced through optimization of HIFU treatment strategies, it may provide a highly desirable adjunct modality against tumor recurrence and metastasis, and thus could improve the overall quality and effectiveness of HIFU therapy for cancers.

In the development of tumor-specific immune responses, antigen presenting cells (APCs), especially dendritic cells (DCs), play a pivotal role [12,13]. In peripheral tissues, DCs capture antigens and migrate to draining lymph nodes, where they present captured antigens and sensitize antigen-specific T-cells to act. To initiate an effective immune response, DCs must be stimulated to undergo a process of maturation, upon which they lose their antigen-acquiring ability, but upregulate the expression of co-stimulatory molecules such as CD80 and CD86, and secrete cytokines including IL-12 and IL-18. These changes in phenotype and function lead to the capability of DC to potently activate T-cells and to induce antigen-specific immune responses [13,14]. On the other hand, unstimulated APCs may also capture antigens but present the captured antigens without costimulation to T-cells, thus causing anergy of T-cells and leading to immune tolerance [15–18].

It is long known that many pathogen-derived signals (e.g., lipopolysaccharides) are potent stimuli for APC activation, which leads to robust immune response against infections [19]. However, it was unclear for some time whether tumor cells can also provide any stimulatory signals for APC, because tumor cells are obviously lacking such pathogen-derived mediators for APC activation. On theoretical ground, Matzinger [20] proposed the ''danger model,'' which hypothesizes that APCs can also be activated by endogenous danger/alarm signals released from distressed or injured cells, thus adding a new category of APC activators. According to this theory, endogenous danger signals are either inducible or exist in a pre-packed form only to be released in situations in which cells are threatened. Although purely theoretical at that time, many endogenous danger signals have since been empirically identified [21]. Among them are ATP [22], UTP [23], heat-shock proteins (hsps) [24,25], and mammalian DNA [26]. Previous studies have demonstrated that APCs can be activated by endogenous danger signals released from tumor cells that are damaged or killed, and the activated APCs could further elicit robust T-cell responses against the tumors [15,27–29]. These studies also suggest that by inducing in situ damage of the tumor, more danger signals together with tumor antigens can be released from tumor cells and a strong anti-tumor immunity may thus be elicited through efficient antigen presentation by the activated DCs.

In this work, the feasibility of utilizing HIFU to produce mechanical lysis of tumor cells with concomitant release of endogenous danger signals or immuno-stimulatory factors (Hsp and ATP) and their stimulatory effects on APCs, especially DCs and macrophages, were investigated. The results demonstrate that endogenous danger signals released from HIFU-treated tumor cells can activate APCs. More importantly, the endogenous danger signals released from mechanically damaged tumor cells were found to be much more potent for APC activation than that from thermally damaged tumor cells, indicating a potential strategy for enhancing anti-tumor immunity during HIFU therapy.

Materials and methods

Cell cultures. RAW264.7 mouse macrophage cell line was obtained from American Type Culture Collection (Manassas, VA). The MC-38 mouse colon adenocarcinoma tumor cell line was kindly provided by Dr. Jeffrey Schlom of NCI (Bethesda, MD). Cell lines were maintained in complete Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, USA) at 37 °C and 5% CO_2. Tumor cells used in the experiments were in log-phase with 24 h doubling time. Before HIFU treatment, tumor cells were washed three times with phosphate-buffered saline (PBS) and the culture medium was replaced with fresh DMEM without FBS. Cell viability before treatment was greater than 98% based on Trypan blue dye exclusion test.

Immature DCs were cultured from the bone marrow of C57BL mice according to a protocol modified from that described by Inaba et al. [30]. Briefly, bone marrow cells were collected from femurs and tibias, resuspended at 1×10^6 cells/ml, and cultured in 50 cm^2 flasks (10 ml/flask) in RPMI-1640 supplemented with 10% FBS, glutamine, β-mercaptoethanol, antibiotics, 10 ng/ml GM-CSF (PharMingen, San Diego, California), and 10 ng/ml IL-4 (BioSource, Camarillo, California). After 2 days incubation at 37 °C, 5% CO_2, non-adherent cells were removed by two washes with pre-warmed (37 °C) RPMI-1640 medium and the adherent cells were cultured in the above complete medium for an additional 3 days. Cells were harvested at day 5 and are immature DCs based upon phenotypic analysis (data not shown).

HIFU treatment. A 1.1 MHz HIFU transducer (H-102, Sonic Concepts, Seattle, WA) with a focal length of 63 mm was mounted horizontally inside a water tank filled with degassed water. As shown in Fig. 1, the HIFU transducer with its 50 Ω matching network was driven by sinusoidal tone-burst signals produced by a function generator (33120 A, Agilent, Palo Alto, CA) together with a 55 dB power

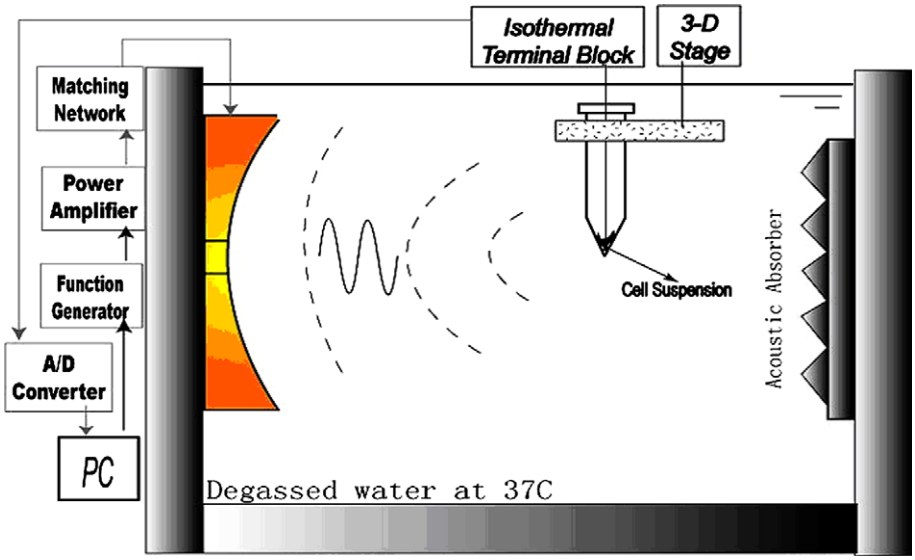

Fig. 1. A schematic diagram of the HIFU experimental system for in vitro cell studies.

amplifier (A150, Electronic Navigation Industries, Rochester, NY). The operation and exposure parameters of the HIFU system were controlled by programs written in LabView (v.6.1, National Instruments, Austin, TX) via a GPIB board installed in a PC. 1×10^6 tumor cells suspended in 10 μl DMEM were loaded in a 0.2 ml PCR thin-walled tube, which was placed vertically with its conical bottom aligned within the −6 dB beam focus of the HIFU transducer. Temperature elevation in cell suspension was monitored using a thin bareware thermocouple (IT-23, Physitemp, Clifton, NJ) during the experiment.

Bioassays for endogenous danger signal release and cellular damage. After HIFU treatment, supernatants of the treated tumor cells were collected by centrifuging the cell suspension at 500 g for 5 min. ATP and hsp60 concentration in the supernatants were determined with ATPLite Luminescence ATP Detection Assay System (Perkin-Elmer, MA, USA) and a commercial ELISA kit (Stressgen Bioreagents, Victoria BC, Canada), respectively. To assess cellular damage, LDH activities in the supernatants of HIFU-treated tumor cell were measured by CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega, WI, USA).

Stimulation of DCs and assay for their maturing status. Immature DCs cultured from mouse bone marrow cells were collected, washed three times with RPMI-1640, suspended at a density of 1×10^6 cells/ml in complete culture medium supplemented with 10 ng/ml GM-CSF and IL-4, and seeded in the wells of a 24-well plate (1 ml/well). The supernatants collected from HIFU-treated tumor cells were then added to the DC cultures. After incubation for 2 days at 37 °C with 5% CO_2, supernatants were harvested and assayed for secreted IL-12 by a commercial ELISA kit (Biosource International, CA, USA). To analyze the expression levels of co-stimulatory molecules, DCs were collected into cold PBS plus 1% dialyzed bovine serum albumin, then washed and stained on ice for 30 min with a combination of the following monoclonal antibodies: APC-Conjugated Anti-Mouse CD11c, FITC-Conjugated Anti-Mouse CD80, and PE-Conjugated Anti-Mouse CD86 (BD Biosciences Pharmingen, USA). Subsequently, the cells were washed again and analyzed using a FACSCalibur flow cytometer (Becton–Dickinson).

Stimulation of macrophages for TNF-α secretion. RAW 264.7 mouse macrophages were seeded at a density of 1×10^6 cells/ml in the wells of a 24-well plate (1 ml/well). The supernatants collected from tumor cells treated with HIFU were then added to the macrophage cultures. After incubation for 6 h at 37 °C with 5% CO_2, supernatants were harvested and assayed for TNF-α levels by ELISA kit (ALPCO Diagnostics, NH, USA).

Statistical analysis. Data were expressed as means ± SEM. Statistical significance was determined by the unpaired two-tailed Student's *t* test.

Results

Thermal and mechanical lysis of the tumor cells produced by a HIFU transducer

Current HIFU therapy is focused almost exclusively on producing thermal ablation of the tumor tissues. However, both thermal and mechanical damage may be caused by HIFU through heat impulse and acoustic cavitation. Here, we compared the HIFU-induced damage of MC-38 tumor cells using two different exposure strategies, with one emphasizing the thermal effect but the other emphasizing the mechanical effect produced by the same 1.1 MHz HIFU transducer under different output conditions. The acoustic field parameters of the HIFU transducer corresponding to these two sets of exposure conditions are given in Table 1. Under the first

Table 1
The output parameters of thermal and mechanical HIFU treatments

Output parameters	Thermal HIFU	Mechanical HIFU
Modulation	Burst mode	Burst mode
Frequency (MHz)	1.1	1.1
PRF (Hz)	200	4
P^+ (MPa)	12.0	31.7
P^- (MPa)	6.7	10.7
Duty cycle (%)	30	3
Treatment duration (s)	5	3×10[a]

PRF, pulse repetition frequency.
[a] At an interval of 10 s.

set of exposure conditions, the HIFU transducer was running at a low pressure level ($P^+ = 12.0$ MPa/$P^- = 6.7$ MPa) with a high duty cycle of 30%, which led to a rapid temperature rise to 80 °C in the cell suspension within a 5 s treatment duration. For convenience, this exposure configuration will be referred to hereafter as "thermal HIFU." In contrast, the second exposure configuration, referred to as "mechanical HIFU," was established by running the HIFU transducer at a high pressure level ($P^+ = 31.7$ MPa/$P^- = 10.7$ MPa) with a low duty cycle of 3%, which led to the production of strong cavitation activity, and thus mechanical stresses, while limiting the temperature rise in the cell suspension to below 40 °C with a 30 s treatment duration.

Microscopy examinations revealed that both the thermal and mechanical HIFU treatments could cause a complete death of the tumor cells, as evidenced by Trypan blue dye incorporation (>99%). However, significant coagulations of morphologically intact cells were observed following thermal HIFU treatment, while extensive cellular fragments and debris were produced by mechanical HIFU treatment (Fig. 2). These results suggest that distinctly different damage patterns can be produced by treatment protocols that emphasize either the thermal or mechanical effects produced by a HIFU transducer.

Cellular damage and release of endogenous danger signals from MC-38 tumor cells treated by thermal and mechanical HIFU

Fig. 3 shows the time course for the release of LDH, a biomarker of cellular damage [31], and the release of endogenous danger signals ATP and hsp60 from MC-38 tumor cells treated by thermal and mechanical HIFU, respectively. In comparison, the mechanical HIFU elicited an immediate and strong release of LDH than the thermal HIFU, suggesting that significantly higher cellular damage was produced by the high pressure and cavitation activities associated with the mechanical HIFU. In addition, the release of hsp60 by the mechanical HIFU was progressive and reached a maximum level almost five times of that produced by the thermal HIFU. Furthermore, the release of ATP was initially high for both thermal and mechanical HIFU-treated groups. However, the ATP released from mechanical HIFU-treated tumor cells broke down quickly, whereas the ATP released from thermal HIFU-treated tumor cells remained stable in the supernatants, indicating the heat-resistance of ATP. Considering that many proteins will denature at temperature above 50 °C, we further tested the heat-stability of LDH and hsp60 by immersing supernatants of mechanical HIFU-treated tumor cells in a water bath of 80 °C for 5 s. The amounts of LDH and hsp60 were found to decrease dramatically after such heat treatment (data not shown). Protein denaturation may also explain the difference in ATP release produced by thermal and mechanical HIFU. Since cells abound with endogenous ATP degrading enzymes (ATPases), these ATPases released from mechanical HIFU-treated tumor cells may rapidly degrade the ATPs that were released simultaneously. In the case of thermal HIFU, since ATPases may be inactivated by heat, the released ATP can remain stable in supernatants. Altogether, these results suggest that thermal and mechanical HIFU can induce distinctively different release profiles of the endogenous danger signals. Thermal HIFU may lead to an incomplete release of heat-resistant danger signals such as ATP but can denature and inactivate heat-sensitive danger signals such as hsp60 as well as endogenous molecule degrading enzymes such as ATPases. On the other hand, mechanical HIFU with its negligible thermal effect together with the strong destructive effect on cell membranes may cause a much more complete release of a diverse array of endogenous danger signals, although some of them may be degraded by simultaneously released endogenous molecule degrading enzymes.

Activation of APCs by danger signals released from mechanical and thermal HIFU-treated tumor cells

To evaluate their propensity in the activation of APCs, supernatants collected from mechanical and

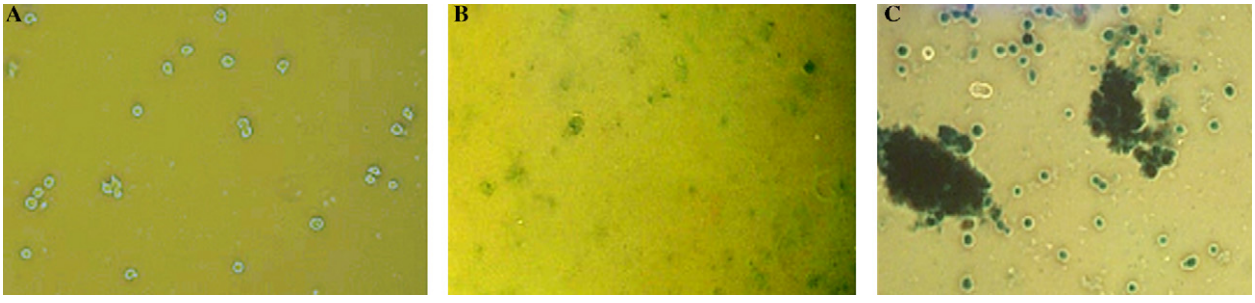

Fig. 2. Microscopy of (A) normal, (B) mechanical HIFU-treated, and (C) thermal HIFU-treated MC-38 tumor cells after Trypan blue dye staining. Complete destruction of the tumor cells was observed following mechanical HIFU, whereas coagulation of morphologically intact tumor cells was observed following thermal HIFU (400× magnification).

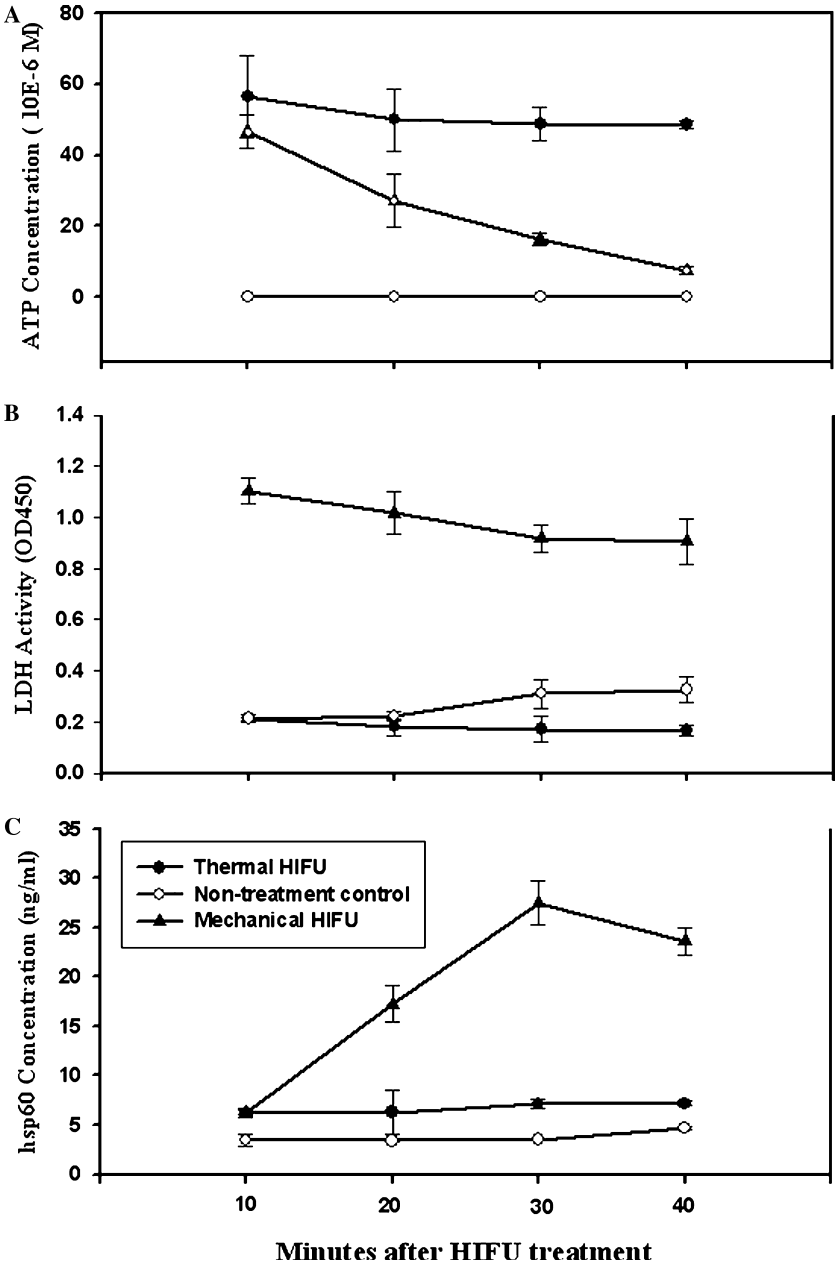

Fig. 3. Release of (A) ATP, (B) LDH, and (C) hsp60 from MC-38 tumor cells treated with either mechanical or thermal HIFU. Data are presented as means ± SEM from triplicate measurements. The results are representative of three independent experiments.

thermal HIFU-treated MC-38 tumor cells, respectively, were assayed for their stimulatory effects on APCs. As shown in Table 2 and Fig. 4, both mechanical and thermal HIFU treatments were found to stimulate the maturation of APCs, as manifested by up-regulation of the expression of co-stimulatory molecules (CD80 and CD86) and enhanced IL-12 secretion from DCs, and significantly elevated TNF-α secretion from RAW264.7 macrophages. More importantly, the stimulatory effect induced by mechanical HIFU treatment was much stronger than that induced by thermal HIFU treatment. Altogether, these findings suggest that the mechanical stresses generated by alternative HIFU treatment strategies are much more effective in releasing immunologically active endogenous danger signals from tumor cells and in the activation of APCs than the thermal stresses produced by the current HIFU cancer therapy.

Discussion

Using tumor cell line MC-38, we have demonstrated in vitro that HIFU treatment can cause both thermal and mechanical necrosis, leading to the release of endogenous danger signals (ATP and hsp60) from the damaged tumor cells. We have further demonstrated that

Table 2
Up-regulated expression of CD80 and CD86, and enhanced secretion of IL-12 by DCs after being exposed to supernatants of MC-38 tumor cells treated with mechanical or thermal HIFU

Exposure group	Mean fluorescence intensity		IL-12 secretion (pg/ml)
	CD80	CD86	
Medium	127 ± 6.1	302 ± 30.2	16.1 ± 3.8
Non-treatment control	128 ± 12.4	308 ± 26.5	40.3 ± 5.0
Mechanical HIFU	154 ± 2.8*	402 ± 8.5*	111.2 ± 8.9**
Thermal HIFU	145 ± 5.0*	372 ± 7.8*	57.9 ± 2.5*

Immature DCs were co-cultured with 0.5 ml supernatant of MC-38 cells that were either treated with mechanical or thermal HIFU. After 48 h, DCs were collected and the expression levels of co-stimulatory molecules in the medium or supernatants of MC-38 cell cultures were assayed by flow cytometry. DCs were identified as the large (FSC hi/SSC hi) CD11c positive population and the surface expression levels of CD80 and CD86 were measured on cells in this population. IL-12 secretion was assayed by collecting DC supernatants and measured for IL-12 by a commercial ELISA kit (Biosource International, CA, USA). The measurements were performed with triplicate samples. Data are shown as means ± SEM.

* $p < 0.05$ compared with non-treatment control.

** $p < 0.001$ compared with non-treatment control and thermal HIFU by Student's t test. The results are representative of three independent experiments.

the released danger signals can stimulate the maturation of APCs, such as DCs and macrophages. Most importantly, the immuno-stimulatory effect of mechanically lysed tumor cells was found to be much stronger than that of thermally ablated tumor cells, which are the predominant outcome of current HIFU therapy. These findings support the notion that HIFU may elicit anti-tumor immunity and point to the possibility of developing alternative HIFU treatment strategies (in contrast to purely thermal ablation) for inducing stronger immune responses against cancer.

It is now well established that the maturation or activation of APCs, especially DCs, plays a critical role in the initiation of the adaptive (or antigen-specific) immune response against cancer [12,13,15]. Based on the "danger model" proposed by Matzinger [20,32], the activation of APCs can be induced by endogenous danger signals released from necrotic cells, such as those exposed to pathogens, toxins, or mechanical damage, but not by healthy cells or those dying apoptotically [15]. In healthy tissues, APCs are kept in a "resting state" and therefore no adaptive immune response is initiated. When distressed or injured, tissues will send out danger signals to alarm APCs to trigger immunity [32]. From this viewpoint, the lack of host immunity against tumors can be explained by the fact that most tumors have evolved to grow without releasing endogenous danger signals, and hence avoiding APC activation and the initiation of adaptive anti-tumor immunity. Further, it has been shown that immature DCs can constitutively capture exosomes from living cells or apoptotic bodies and present the captured antigens to T-cells. Without co-stimulation, however, T-cells presented with antigen will either enter a state of anergy or be deleted [12,33]. To break such a barrier of immune tolerance for tumors, a conceptually attractive approach is the induction of necrotic damage to tumor cells, which leads to the release of endogenous danger signals together with plentiful tumor antigens that can activate APCs to induce effective T-cell immunity.

Following this conceptual lead, various treatment strategies for enhancing anti-tumor immunity have been explored. For example, tumor cells that are

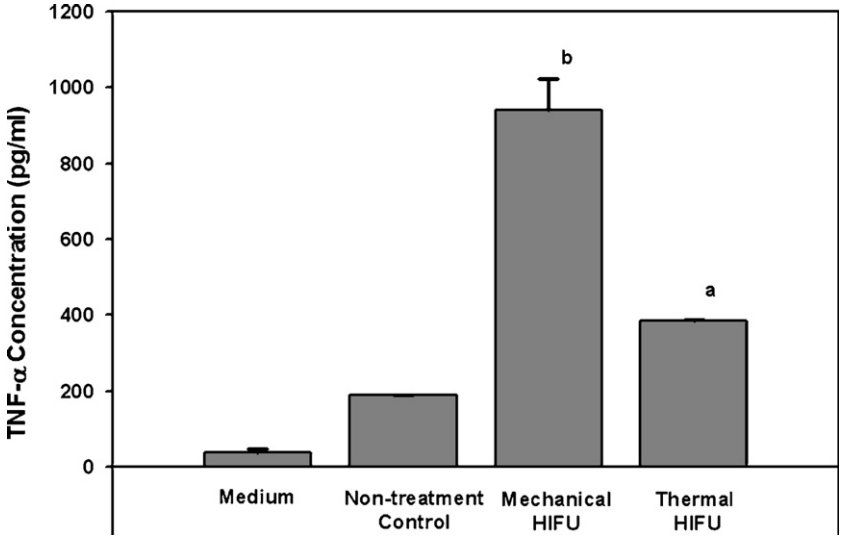

Fig. 4. Enhanced secretion of TNF-α by RAW264.7 mouse macrophages after exposure to supernatants of MC-38 tumor cells treated by HIFU. The measurements were performed with triplicate samples. Data are shown as means (±SEM). [a]$p < 0.05$ compared with non-treatment control, [b]$p < 0.001$ compared with non-treatment control and thermal HIFU by Student's t test. The results are representative of three independent experiments.

mechanically lysed by repeated freezing and thawing have been used to provide strong stimuli for APC activation [15,28,34]. This approach, although convenient for in vitro studies, has limitations for in vivo applications. Others have examined the response of APCs to tumor cells that are treated by chemotherapeutic agents and/or radiation, which mainly cause tumor cells to undergo apoptosis. The results are controversial. Some studies suggest that such treatments fail to provide a stimulatory signal for APCs or may even block the maturation of DCs [15,28,34]. Yet, other studies suggest that chemotherapeutic agents and radiation could cause tumor cells to undergo secondary necrosis in addition to apoptosis, and therefore stimulatory signals may be released to activate APCs [29]. Nevertheless, the most significant drawback of chemotherapy and radiation is their cytotoxic effects on hemopoietic cells of the bone marrow and lymphocytes of the immune system, leading to immuno-suppression. This side effect may severely limit their potential to augment anti-tumor immunity. In contrast, unlike radiation and chemotherapy, HIFU can be applied repetitively without the apprehension of accumulating systemic toxicity. This unique feature, combined with the localized destruction of tumor cells with concomitant release of endogenous danger signals, makes HIFU an ideal strategy for potentially eliciting a strong anti-tumor immunity.

Previous animal and clinical studies have provided preliminary evidence suggesting that HIFU treatment may elicit a systemic anti-tumor immune response [6,9–11]. However, two fundamental issues that are critical for enhancing the therapeutic effect of HIFU-elicited anti-tumor immunity were not addressed. First, the underlying immunological mechanism for HIFU-induced anti-tumor immunity was not investigated. Without this knowledge, it is difficult to improve the potency of anti-tumor immunity elicited by HIFU. Second, the anti-tumor immune response induced in the previous HIFU studies, if any, was not strong enough to provide a complete protection against tumor recurrence or metastasis. In fact, local tumor recurrence and metastasis are often the cause of failure for HIFU therapy [7], indicating the need to augment the host anti-tumor immunity.

Based on the results of our in vitro experiments and Matzinger's "danger model" theory, we postulate that the release of endogenous danger signals from HIFU-damaged tumor cells and the consequent activation of APCs may constitute an important mechanism for HIFU-elicited anti-tumor immunity. The observation of lower potency in the stimulatory effect of DC maturation by thermal HIFU (coagulative necrosis) as compared to mechanical HIFU (disruptive necrosis) is also important. These findings suggest that current HIFU therapy, which produces predominantly coagulative necrosis by thermal stresses, may have two serious shortcomings in eliciting a strong anti-tumor immunity. First, coagulative necrosis may lead to an incomplete release of the endogenous danger signals (see Fig. 2C). Second, even when some of the endogenous danger signals were released, their potency could be severely damaged by the thermal stresses, as demonstrated by the inactivation of heat-sensitive hsp60 following thermal HIFU treatment (see Fig. 3). In comparison, when HIFU was used to produce predominantly mechanical lysis of the tumor cells without the thermal stress, the release of the endogenous danger signals was more plentiful, presumably due to complete disruption of the cell membranes (see Fig. 2B), and their overall potency in stimulating the maturation of APCs was significantly higher (see Table 2 and Fig. 4).

In recent years, HIFU has been used successfully in clinic for the treatment of a variety of cancers. In its current form, however, HIFU therapy is not effective against metastatic or even residual tumor cells at the primary tumor site. This is partially due to the fact that current HIFU therapy is used almost exclusively to produce thermal ablation of the tumor while largely ignoring the biological effects that can be produced by the mechanical stresses imposed by focused ultrasound in tissue. By exploring the response of tumor tissues to the mechanical stresses induced by focused ultrasound, and by synergistic combination of HIFU-elicited anti-tumor immunity and thermal ablation through optimization of treatment strategies, the overall quality and effectiveness of cancer therapy by HIFU may be significantly improved in the future.

Acknowledgment

This work was supported in part by NIH Grant No. RO1-EB02682.

References

[1] J.E. Kennedy, G.R. TerHaar, D. Cranston, High intensity focused ultrasound: surgery of the future, Br. J. Radiol. 76 (2003) 590–599.
[2] L. Chen, G.R. terHaar, C.R. Hill, S.A. Eccles, G. Box, Treatment of implanted liver tumors with focused ultrasound, Ultrasound Med. Biol. 24 (1998) 1475–1488.
[3] Z.B. Wang, F. Wu, Z.L. Wang, Z. Zhang, J.Z. Zou, C. Liu, Y.G. Liu, G. Cheng, Y.H. Du, Z.C. He, M.L. Gu, Z.G. Wang, R. Feng, Targeted damage effects of high intensity focused ultrasound (HIFU) on liver tissues of Guizhou Province miniswine, Ultrason. Sonochem. 4 (1997) 181–182.
[4] R. Yang, C.R. Reilly, F.J. Rescorla, P.R. Faught, N.T. Sanghvi, F.J. Fry, T.D. Franklin Jr., L. Lumeng, J.L. Grosfeld, High-intensity focused ultrasound in the treatment of experimental liver cancer, Arch. Surg. 126 (1991) 1002–1009.
[5] C. Chaussy, S. Thuroff, The status of high-intensity focused ultrasound in the treatment of localized prostate cancer and the impact of a combined resection, Curr. Urol. Rep. 4 (3) (2003) 248–252.
[6] X. Wang, J. Sun, High-intensity focused ultrasound in patients with late-stage pancreatic carcinoma, Chin. Med. J. (Engl.) 115 (9) (2002) 1332–1335.

[7] F. Wu, Z.B. Wang, W.Z. Chen, J.Z. Zhou, J. Bai, H. Zhu, K.Q. Li, F.L. Xie, C.B. Jin, H.B. Su, G.W. Gao, Extracorporeal focused ultrasound surgery for treatment of human solid carcinomas: early Chinese clinical experience, Ultrasound Med. Biol. 30 (2004) 245–260.
[8] G.J. Van Leenders, H.P. Beerlage, E.T. Ruijter, J.J. de la Rosette, C.A. van de Kaa, Histopathological changes associated with high intensity focused ultrasound (HIFU) treatment for localised adenocarcinoma of the prostate, J. Clin. Pathol. 53 (5) (2000) 391–394.
[9] F. Wu, Z.B. Wang, P. Lu, Z.L. Xu, W.Z. Chen, H. Zhu, C.B. Jin, Activated anti-tumor immunity in cancer patients after high intensity focused ultrasound ablation, Ultrasound Med. Biol. 30 (9) (2004) 1217–1222.
[10] D.F. Rosberger, D.J. Coleman, R. Silverman, S. Woods, M. Rondeau, S. Cunningham-Rundles, Immunomodulation in choroidal melanoma: reversal of inverted CD4/CD8 ratios following treatment with ultrasonic hyperthermia, Biotechnol. Ther. 5 (1-2) (1994) 59–68.
[11] R. Yang, C.R. Reilly, F.J. Rescorla, et al., Effects of high-intensity focused ultrasound in the treatment of experimental neuroblastoma, J. Pediatr. Surg. 27 (1992) 246–251.
[12] M.J. Smyth, D.I. Godfrey, J.A. Trapani, A fresh look at tumor immunosurveillance and immunotherapy, Nat. Immunol. 2 (2001) 293–299.
[13] J. Banchereau, R.M. Steinman, Dendritic cells and the control of immunity, Nature 388 (1997) 787–792.
[14] M. Cella, F. Sallusto, A. Lanzavecchia, Origin, maturation and antigen presenting function of dendritic cells, Curr. Opin. Immunol. 9 (1997) 10–16.
[15] S. Gallucci, M. Lolkema, P. Matzinger, Natural adjuvants: endogenous activators of dendritic cells, Nat. Med. 5 (1999) 1249–1255.
[16] C. Kurts et al., Constitutive classI-restricted exogenous presentation of self antigen in vivo, J. Exp. Med. 184 (1996) 923–930.
[17] J.P. Ridge, E.J. Fuchs, P. Matzinger, Neonatal tolerance revisited: turning on newborn T cells with dendritic cells, Science 271 (1996) 1723–1726.
[18] C. Harding, J. Heuser, P. Stahl, Endocytosis and intracellular processing of transferrin and colloidal gold-transferrin in rat reticulocytes: demonstration of a pathway for receptor shedding, Eur. J. Cell Biol. 35 (1984) 256–263.
[19] R. Medzhitov, C.A. Janeway Jr., Innate immunity: the virtues of a nonclonal system of recognition, Cell 91 (3) (1997) 295–298.
[20] P. Matzinger, Tolerance, danger, and the extended family, Annu. Rev. Immunol. 12 (1994) 991–1045.
[21] S. Gallucci, P. Matzinger, Danger signals: SOS to the immune system, Curr. Opin. Immunol. 13 (2001) 114–119.
[22] M. Schnurr, F. Then, P. Galambos, C. Scholz, B. Siegmund, S. Endres, A. Eigler, Extracellular ATP and TNF-alpha synergize in the activation and maturation of human dendritic cells, J. Immunol. 165 (2000) 4704–4709.
[23] I. Marriott, E.W. Inscho, K.L. Bost, Extracellular uridine nucleotides initiate cytokine production by murine dendritic cells, Cell. Immunol. 195 (2) (1999) 147–156.
[24] R.P. Wallin, A. Lundqvist, S.H. More, A. von Bonin, R. Kiessling, H.G. Ljunggren, Heat-shock proteins as activators of the innate immune system, Trends Immunol. 23 (3) (2002) 130–135.
[25] W. Chen, U. Syldath, K. Bellmann, V. Burkart, H. Kolb, Human 60-kDa heat-shock protein: a danger signal to the innate immune system, J. Immunol. 162 (6) (1999) 3212–3219.
[26] K.J. Ishii, K. Suzuki, C. Coban, F. Takeshita, Y. Itoh, H. Matoba, L.D. Kohn, D.M. Klinman, Genomic DNA released by dying cells induces the maturation of APCs, J. Immunol. 167 (5) (2001) 2602–2607.
[27] S. Buttiglieri, A. Galetto, S. Forno, M. De Andrea, L. Matera, Influence of drug-induced apoptotic death on processing and presentation of tumor antigens by dendritic cells, Int. J. Cancer 106 (4) (2003) 516–520.
[28] B. Sauter, M.L. Albert, L. Francisco, et al., Consequences of cell death: exposure to necrotic tumor cell, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells, J. Exp. Med. 19 (2000) 423–433.
[29] Y. Shi, W. Zheng, K.L. Rock, Cell injury releases endogenous adjuvants that stimulate cytotoxic T cell responses, Proc. Natl. Acad. Sci. USA 97 (26) (2000) 14590–14595.
[30] K. Inaba, M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, R.M. Steinman, Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor, J. Exp. Med. 176 (6) (1992) 1693–1702.
[31] M. Drent, N.A. Cobben, R.F. Henderson, E.F. Wouters, M. van Dieijen-Visser, Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation, Eur. Respir. J. 9 (8) (1996) 1736–1742.
[32] P. Matzinger, The danger model: a renewed sense of self, Science 296 (5566) (2002) 301–305.
[33] D.M. Pardoll, Spinning molecular immunology into successful immunotherapy, Nat. Rev. Immunol. 2 (2002) 227–238.
[34] S. Basu, R.J. Binder, R. Suto, K.M. Anderson, P.K. Srivastava, Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway, Int. Immunol. 12 (11) (2000) 1539–1546.