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● *Original Contribution*

ACTIVATION OF TUMOR-INFILTRATING ANTIGEN PRESENTING CELLS BY HIGH INTENSITY FOCUSED ULTRASOUND ABLATION OF HUMAN BREAST CANCER

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Abstract—Previous studies have shown that high intensity focused ultrasound (HIFU) ablation can trigger activation of host antitumor responses after direct tumor destruction. The goal of this study was to investigate the status and functions of tumor-infiltrating antigen presenting cells (APCs) after HIFU ablation of human breast cancer, and to explore the mechanisms regarding HIFU-enhanced antitumor response. Forty-eight women with biopsy-proven breast cancer were divided randomly into a control group ($n = 25$) and a HIFU group ($n = 23$). Patients in the control group received modified radical mastectomy, and those in the HIFU group underwent HIFU ablation of primary breast cancer, followed by modified radical mastectomy within 1–2 weeks. Using immunohistochemical analysis, tumor-infiltrating dendritic cells (DCs), macrophages, B lymphocytes and expression of HLA-DR and costimulatory molecules on DCs and macrophages were assessed in all patients. The results showed that APCs infiltrated along the margins of the ablated regions in all HIFU-treated tumors, and numbers of tumor-infiltrating DCs, macrophages and B lymphocytes increased significantly in the HIFU group. Compared with the values in the control group, the percentage of DCs and macrophages expressing HLA-DR, CD80 and CD86 was significantly greater in the HIFU group. There were statistically significant differences between numbers of S-100$^+$ HLA-DR$^+$, S-100$^+$ CD80$^+$, S-100$^+$ CD86$^+$, CD68$^+$ HLA-DR$^+$, CD68$^+$ CD80$^+$ and CD68$^+$ CD86$^+$ cells in the control and HIFU groups, respectively. It was concluded that HIFU ablation induces significant infiltration of APCs within the residual tumor debris in patients with breast cancer, and most of the tumor-infiltrating DCs and macrophages were activated after HIFU ablation. (E-mail: mfengwu@yahoo.com)

Key Words: **High-intensity focused ultrasound, Breast cancer, Antigen presenting cell, Dendritic cell, Antitumor immunity, Thermal ablation.**

INTRODUCTION

Antigen presenting cells (APCs) play a central role in immune responses against tumors. APCs can infiltrate local tumors and present tumor antigens to naïve T lymphocytes in a MHC restricted fashion. Activating signals, delivered directly or indirectly by tumor cells, can induce the progression of infiltrating APCs from an immature to a mature stage. During maturation, APCs increase the expression of costimulatory molecules, such as CD80 and CD86, and become efficient in a process of cross-priming T cells (Lutz and Schuler 2002). However, the absence or blockade of these costimulatory molecules impairs tumor antigen-specific immune responses, indicating that antitumor immunity requires the activation of APCs (Pinzon-Charry et al. 2005).

The transfer of tumor antigens from APCs to T lymphocytes is a key event for the initiation of lymphocyte-mediated immunity against tumors. APCs include dendritic cell (DCs), macrophages and B lymphocytes, and DCs are recognized as the most potent APCs in the process of uptake, processing and presentation of tumor antigens. The presence of DCs with positive expression of activated markers has been reported in various human tumors (Enk et al. 1997; Lespagnard et al. 1999; Scarpino et al. 2000; Tsujitani et al. 1990; Zeid and Muller 1993), and most studies have revealed that tumor-infiltrating DCs were a positive prognostic indicator in cancer patients (Pinzon-Charry et al. 2005; Vicari et al. 2002; Yang and Carbone 2004).

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As a noninvasive therapy, high-intensity focused ultrasound (HIFU) ablation has recently received increasing interest in the treatment of human malignancies (Chaussy et al. 2005; Kennedy 2005; Wu 2006). Previous studies have shown that HIFU can activate host antitumor responses after direct tumor destruction, and this enhancement has the potential to help the host immune system to control micro-metastases and to decrease local recurrence (Rosberger et al. 1994; Wang and Sun 2002; Wu et al. 2004; Yang et al. 1992). Large amounts of ablated tumor debris contain various tumor antigens, and these antigens can be exposed *in situ* and reabsorbed after HIFU ablation (Wu et al. 2007a). The most striking change seen in the tumor debris was the upregulated expression of heat shock proteins (HSPs), such as HSP60 (Hu et al. 2005), HSP27 (Madersbacher et al. 1998), HSP72 and HSP73 (Kramer et al. 2004) and HSP70 (Wu et al. 2007b), suggesting that HIFU ablation could increase tumor immunogenicity. HSPs are intracellular molecular chaperones, and APCs take up HSP-tumor peptide complex and present the chaperoned peptides directly to tumor-specific T-cells, resulting in potent cellular immune responses against tumors (Pockley 2003). However, it has remained unclear whether APCs actually infiltrate the ablated tumors after HIFU ablation, and whether these cells could be activated locally to initiate antigen-specific immune responses. In this study, we hypothesized that tumor-infiltrating APCs would be activated after HIFU ablation compared with surgical resection in patients with breast cancer. Thus, the goal of this study was to investigate the status and function of tumor-infiltrating APCs after HIFU ablation of breast cancer in a randomized clinical trial, and to explore the possible mechanisms behind HIFU-enhanced antitumor response.

MATERIALS AND METHODS

Patients

All patients referred to in this study were recruited into a randomized clinical trial of HIFU treatment for breast cancer. The trial was approved by the ethics committee at Chongqing Medical University, and each patient signed an informed consent form at the time of enrollment in accordance with the specification stipulated by the Helsinki Committee. Details of the trial have been described previously in the published literature (Wu et al. 2003). Briefly, 48 women with biopsy-proven breast cancer were divided randomly into a control group ($n = 25$) and a HIFU group ($n = 23$). Patients in the control group received modified radical mastectomy, and those in the HIFU group underwent HIFU ablation of breast cancer, followed by modified radical mastectomy within 1–2 weeks. A comparison of the eligible patients' characteristics in both groups is shown in Table 1.

Table 1. The characteristics of enrolled patients with breast cancer in both groups

Characteristics	Control group	HIFU group
No. of patients	25	23
Age (y)	45.5 ± 1.2	46.5 ± 1.7
Tumor diameter (cm)	3.5 ± 0.23 (1.8–5.6)	3.1 ± 0.79 (2.0–4.7)
Histology		
Infiltrating duct	19	21
Lobular carcinoma in situ	2	2
Medullary	2	0
Mucinous	2	0
Node status		
N0	13	12
N1	6	5
N2	6	6
TNM stage		
I	2	2
II	22	21
III	1	0

HIFU treatment

An ultrasound-guided HIFU system (Model-JC, Chongqing Haifu Tech Co., Ltd, China) was used in this study to treat 23 patients with breast cancer. The HIFU device and treatment procedure has been described in detail previously (Illing et al. 2005; Wu et al. 2003). The therapeutic transducer operated at a frequency of 1.6 MHz, and acoustic focal peak intensities ranged from 5,000–15,000 W cm^{-2}. All patients received one session of HIFU treatment directed at their primary breast cancer. The ablated extent included the breast lesion and 1.5–2.0 cm of normal breast tissue surrounding the visible tumor. Mean HIFU exposure time was 1.3 h (range 45 min to 2.5 h).

Tissue specimens

Modified radical mastectomy was performed in all of the eligible patients, and each excised breast was immediately collected. The breast specimens included breast tumor and normal breast tissue. Representative sections were prepared for routine microscopic analysis. Tissue blocks were sampled from the central to peripheral regions of tumors (in the control group) or the treated region (in the HIFU group). They were fixed in 10% phosphate-buffered formalin (pH = 7), embedded in paraffin and cut into 4-μm-thick slices for immunohistochemistry examination.

Immunohistochemical staining

The primary antibodies used in this study are listed in Table 2. By using standard biotin-streptavidin-perox-