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## High intensity focussed ultrasound (HIFU) acts as immunomodulatory against cancer: A review

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**Abstract**

Focused ultrasound has been widely acclaimed to be and used by many to generate highly localized heating to treat cysts and cancerous tumours through a process called Magnetic Resonance guided Focused Ultrasound (MRgFUS) or High Intensity Focused Ultrasound (HIFU). These procedures guided by Magnetic Resonance Imaging (MRI) generally works on lower frequencies than medical diagnostic ultrasound with significantly higher energies. By tweaking, the parameters of the focused ultrasound treatment can induce the immune response through enhanced dendritic cell (DCs) infiltration and activation. Dendritic cells can capture and present activating signals delivered by necrotic tumour cells that remain in-situ after HIFU treatment. The other beneficial side effects of HIFU leads to higher CD4+/CD8+ ratio and increasing CD3+, CD4+, and CD8+ lymphocytes in the circulation. HIFU based therapy ultrasound shown to potentially boost the body's immune response thus combating the local recurrence of the targeted tumour.

**Keywords:** Cancer immunotherapy, dendritic cells, cytotoxic t-lymphocytes, HIFU

**Introduction**

High intensity focused ultrasound (HIFU) is a hyperthermia treatment, which is a class of clinical therapies that uses high temperature to treat diseases. HIFU is also one method of therapeutic ultrasound, non-invasive methods to direct acoustic energy into the body (Robertson and Bakers, 2001) [19]. The induction of an effective antitumor immune response is dependent on dendritic cells (DCs), antigen-presenting cells (APCs) that capture antigens and migrate to secondary lymphoid organs, where they present the captured antigens to T cells and activate them. Some evidence are showing that HIFU can stimulate DCs to infiltrate into tumour tissues, migrate to draining lymph nodes after being activated, and subsequently elicit tumour specific CTL responses. Previously postulated that in situ activation of DCs inside HIFU-treated tumour tissue constitute mechanism for HIFU-induced anti-tumour immunity. The central role of DCs maturation in the development of anti-tumour immune response and optimized HIFU strategy that can effectively activate DCs to mature should have potential to elicit a stronger anti-tumour immunity.

**Efficacy of HIFU in cancer immunotherapy**

Of late, HIFU has emerged as a viable alternative to surgical resection of solid tumours, morbidity, hospital stay, cost, and improved quality of life for cancer patients (Wu, 2013) [24]. However, focused ultrasound has also been shown to potentially boost the body's immune response. HIFU is one of the potential tool as an agent of immunotherapy used extensively in cancer therapy as its non-invasiveness, use of non-ionizing radiation. HIFU could be more appealing for this combination therapy than other ablative modalities (Table.1).

**Role of dendritic cells in tumour immunology**

Dendritic cells (DCs) are stimulators of B and T lymphocytes. T lymphocytes need the antigen to be processed and presented to them by an Antigen Presenting Cells (APC). The T-cell antigen receptors (TCRs) recognize of antigens bound to molecules of the major histocompatibility complex (MHC) on the surface of an APC. The peptide-binding proteins MHC-I, which stimulate cytotoxic T cells (CTLs). Intracellular antigens, cut into peptides in the cytosol of the APC, bind to MHC-I molecules and are recognized by CTLs.

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Once activated, directly that can kill a target cell (Khokhlova and Hwang, 2011), (Banchereau & Steinman, 1998)<sup>[9]</sup>.

DCs considered as the best antigen-presenting cells that can target most of the naive T cells, and plays a crucial role in the generation of tumour specific CTLs. DCs are identified with the expression of CD11c as a cell surface marker. Generally the ratio or the proportions of CD11c cells significantly increase in spleens to more than 3-fold and 1.2-fold than the control group without HIFU treatment (Palucka and Banchereau, 2013)<sup>[18]</sup>. The proportion of CD11c cells at the time point of 2 days after HIFU treatment, but it is too early to observe an increase in the DC population. HIFU treatment enhances the expression of co-stimulatory molecule, CD80 and CD86, on DCs. HIFU treatment appears to enhance recruitment and expansion of DCs, in addition to DC maturation (Tim *et al.*, 2013).

Wu (2004)<sup>[23]</sup> had found that the focused ultrasound shown to potentially boost the body's immune response, which can help combat metastases. HIFU ablation increased the cytotoxicity of cytotoxic T-lymphocytes (CTL) and increase of IFN- $\gamma$  and TNF- $\alpha$  secretion. The frequency of the MHC-I and CD8+ cells was significantly higher in the HIFU group. Inhibition of tumour progression and higher survival rates were observed as adoptive immunotherapy. (Zhou, 2014).

### Dendritic cells and the control of immunity

In situ distribution to optimize antigen capture migration into lymphoid organs to optimize clonal selection: Capacity of less numbers of DCs and low levels of antigen to induce strong T-cell responses (Wang and Sun, 2012). TNF- $\alpha$  and IFN- $\gamma$  secretion of cytotoxic T lymphocytes against H22 cells were significantly higher in HIFU-ablated tumour group than tumour lysate group. HIFU found to activate a systemic anti-tumour immunity and may subsequently augment host antitumor immunity (Tiong and Maddern, 2011)<sup>[21]</sup>.

Several workers had proved an enhanced potency of dendritic cell infiltration and activation (Liu *et al.*, 2010). Liu *et al.* (2012)<sup>[14]</sup> also demonstrated that using low-pressure, pulse-mode focused ultrasound in the presence of microbubbles can elicit an anticancer immune response. Studies have revealed that tweaking the parameters of the focused ultrasound treatment can enhance the immune response.

What is it that makes a DC such a good APC First, they can take up particles and microbes by phagocytosis. Second, they scan large vesicles in which extracellular fluid and solutes are sampled, a process called macropinocytosis.

They express receptors that mediate endocytosis, including C-type lectin receptors like the macrophage mannose receptor (Ali *et al.*, 2005)<sup>[1]</sup>. Macropinocytosis and receptor-mediated antigen uptake make antigen presentation so efficient that small concentrations of antigen. Once the DC has captured an antigen, which also can provide a signal to mature and time to assemble antigen-MHC-II complexes. The antigen enters the endocytic pathway of the cell. In macrophages most of the protein substrate is directed to the lysosomes, an organelle with only few MHC-II molecules, where the antigen is fully digested into amino acids (Deng, 2010)<sup>[6]</sup>. Not in DCs; the DC is able to produce large amounts of MHC-II-peptide complexes at a stage of its life.

As soon as an antigen instructs the DCs to move into gear, fragments of antigen are loaded onto MHC-II molecules and these complexes are sent to the cell surface (Rosberger, 1994)<sup>[20]</sup>. To generate cytotoxic killer cells, which have the capacity to attack tumour cells, DCs have to present antigenic peptides complexed to MHC-I molecules to CD8+ expressing T cells.

A dedicated peptide transporter then translocate from the cytosol to the endoplasmic reticulum, where they bind to MHC-I molecules. The peptide-loaded MHC-I complexes travel to the cell surface where they are displayed for scrutiny by T cells. Nevertheless, they can present peptides from dying infected cells by MHC-I efficiently if they were infected themselves (Blazickova, *et al.* 2000)<sup>[4]</sup>. By processing dying cells, DCs may be able to cross-prime T cells to another cell antigens or self-proteins, which could have major clinical implications.

### Effect of HIFU on cytotoxic T-lymphocytes

It was observed that tumour progression was completely suppressed by both 0.6 and 1.4-MPa microbubble-enhanced HIFU with infiltration of non-T regulatory (non-Treg), tumour infiltrating lymphocytes (TILs) and infiltration of CD8+ cytotoxic T-lymphocytes (CTL). The ratio of CD8+ and Treg increased significantly and tumour growth was inhibited. HIFU induced local enhancement of microvascular permeability within a tumour (Formenti and Demaria, 2009)<sup>[7]</sup>. Similarly, the microbubble-enhanced HIFU is increasing host antitumor immune response and provides local modulation of the immune environment. But long exposure of ultrasound (0.66 W/cm<sup>2</sup>; 7–60 minutes) did not affect cell viability and was associated with only a slight increase in apoptotic keratinocytes and dendritic cells. It could have triggered an immune cell response through cellular damage of tumour cells in the absence of microbubbles. Presence of microbubbles (MBs) enhances blood-vessel permeability, changing the microenvironment of the tumour tissue region, enhancing the recruitment and penetration of tumour-infiltrating lymphocytes into the tumour tissue (Liu *et al.*, 2012)<sup>[14]</sup>.

Another possible mechanism to trigger the production of heat-shock protein (HSP) or other immunomodulatory factors (danger signals) which could in turn trigger TIL infiltration (Joseph, 2009)<sup>[8]</sup>. It was also demonstrated that hsp70 over expression with intensity = 53 W/cm<sup>2</sup> and 1 second of ultrasound exposure without temperature elevation (Basu and Srivastava, 2003)<sup>[2]</sup>. The hsp60 increased in regions of HIFU suggesting that induction of heat shock protein over expression may be involved in triggering of the immune response by microbubble-enhanced HIFU. Besides these, vascular disruption caused by HIFU may also be involved in tumour suppression (Xing *et al.*, 2008)<sup>[26]</sup>. Some previous reports showed that direct damage of the tumour endothelium will initiate vascular collapse, shut down tumour blood flow, and induce ischemic neoplastic cell death. Strategies aimed to target cytokine induction (Xia *et al.*, 2012)<sup>[25]</sup>.

The immune-triggering effects of high-pressure HIFU have been studied for nearly two decades (Hansler, 2006)<sup>[12]</sup>. HIFU thermal ablation of osteosarcoma, hepatocellular carcinoma, and renal cell carcinoma was found to be accompanied by a marked increase in CD4+ cells at the ablated tumour tissue margins, but no significant changes in CD8+ or CD3+ cell populations. The thermal tumour tissue destruction caused by HIFU produces tumour debris antigens that attract infiltration of numerous leukocytes at the margins of the coagulated tissues, and the immune response is thus primarily mediated by cell debris and the accompanying inflammatory reaction (Berge *et al.*, 2010)<sup>[3]</sup>.

More recently, mechanical pulsed-HIFU and tumour-cell destruction has been shown to elicit an antitumor immune response. Hu *et al.*, 2012 demonstrated that increased release of endogenous ATP and hsp60 triggers secretion of

interleukin-12 by DCs and TNF-alpha (Liu *et al*, 2012)<sup>[14]</sup>.

### Conclusions and future directions

In conclusion, impact of HIFU exposure that results in APC-presenting and macrophage-recruiting immunological changes and a new opportunity to facilitate alterations in a tolerogenic tumour microenvironment, possibly serving as an independent therapeutic route by cytotoxic T-lymphocytes or an effective adjuvant for current first-line cancer treatment. Future

research still needs to be done to optimize the HIFU treatment method, find other combinational therapies with dendritic cells and cytotoxic T-lymphocytes, and to further investigate the cause and extent of the antitumor response associated with cytotoxic T-lymphocytes.

**Competing interests:** None of the authors have any competing interests.

**Table 1:** Immunological effects after HIFU

S.No	Articles	Immunologic effects of focused ultrasound
1.	Yang <i>et al.</i> (1992)	Resistance to tumor re-challenge
2.	Rosberger <i>et al.</i> (1994) <sup>[20]</sup>	2/3 patients reverted CD4+/CD8+ ratio from abnormal levels
3.	Madersbacher <i>et al.</i> (1998)	Increase in HSP27
4.	Wang and Sun (2002) <sup>[22]</sup>	Increased CD3+ & CD4+ cells and CD4+/CD8+ ratio in 10 patients, not significant. NK cell activity was significantly enhanced
5.	Kramer <i>et al.</i> (2004)	Up-regulated expression of HSP72, HSP73, GRP75 and GRP78. Increased release of IL-2, IFN-gamma, and TNF-alpha. Decreased release of IL-4, -IL-5, and IL-10
6.	Wu <i>et al.</i> (2004) <sup>[23]</sup>	Increased CD4+ T cells and CD4+/CD8+ ratio
7.	Hu <i>et al.</i> (2005)	ATP and HSP60 is released from tumor cells. Activation of DCs and macrophages. Enhanced IL-12 and TNF-alpha secretion. Mechanical activation of APCs is stronger than thermal
8.	Hu <i>et al.</i> (2007)	Mechanical FUS better at activating DCs than Thermal FUS. Resistance to tumor re-challenge. Increased CTL activity and tumor-specific IFN-gamma-secreting cells
9.	Hundt <i>et al.</i> (2007) <sup>[13]</sup>	HSP70 expression can be induced at lower temperatures than heat stress alone
10.	Xing <i>et al.</i> (2008) <sup>[26]</sup>	Increased CTL cytotoxicity. No increased risk of metastasis after HIFU treatment

### References

- Ali MY. Activation of dendritic cells by local ablation of hepatocellular carcinoma. *J Hepatol.* 2005; 43:817-822.
- Basu S, Srivastava PK. Fever-like temperature induces maturation of dendritic cells through induction of hsp90. *Int Immunol.* 2003; 15(9):1053-61. [PubMed: 12917257]
- Berge V, Baco E, Karlsen SJ. A prospective study of salvage highintensity focused ultrasound for locally radiorecurrent prostate cancer: early results. *Scand J Urol Nephrol.* 2010; 44:223-7.
- Blazickova S *et al.* Effect of hyperthermic water bath on parameters of cellular immunity. *Int J Clin Pharmacol Res.* 2000; 20(1-2):41-6. [PubMed: 11146901]
- Christian Chaussy G, Stefan Thüroff F. Robotic High-intensity Focused Ultrasound for Prostate Cancer: What Have We Learned in 15 Years of Clinical Use?, *Curr Urol Rep*, 2011. DOI 10.1007/s11934-011-0184-2
- Deng J, Zhang Y, Feng J, Wu F. Dendritic Cells Loaded with Ultrasound-Ablated Tumour Induce *in vivo* Specific Antitumour Immune Responses. *Ultrasound in Medicine & Biology.* 2010; 36(3):441-448.
- Formenti SC, Demaria S. Systemic effects of local radiotherapy. *Lancet Oncol.* 2009; 10:718-726.
- Joseph Skitzki J, Elizabeth Repasky A, Sharon Evans S. Hyperthermia as an immunotherapy strategy for cancer, *Curr Opin Investig Drugs.* 2009; 10(6):550-558.
- Jacques Banchereau, Ralph Steinman M. Dendritic cells and the control of Immunity Immune-regulatory effects, *Nature.* 1998; 392:245-252.
- Jian Deng, Ying Zhang, Jun Feng, Feng Wu. Dendritic Cells Loaded with Ultrasound-Ablated Tumour Induce *in vivo* Specific Antitumour Immune Responses, *Ultrasound Medicine.* 2010; 36(3):441-448. DOI: <http://dx.doi.org/10.1016/j.ultrasmedbio>.
- Haen SP, Pereira PL, Salih HR, Rammensee H, Gouttefangeas C. More than Just Tumor Destruction: Immunomodulation by Thermal Ablation of Cancer. *Clinical and Developmental Immunology*, 2011, 1-19.
- Hansler J, Wissniowski TT, Schuppan D, Witte A, Bernatik T, Hahn EG *et al.* Activation and dramatically increased cytolytic activity of tumor specific Tlymphocytes after radio-frequency ablation in patients with hepatocellular carcinoma and colorectal liver metastases. *World J Gastroenterol.* ed. 2006; 12:3716-21.
- Hundt W, O'Connell-Rodwell CE, Bednarski MD, Steinbach S, Guccione S. *In vitro* effect of focused ultrasound or thermal stress on HSP70 expression and cell viability in three tumor cell lines. *Acad. Radiol.* 2007; 14:859-870.
- Liu H, Hsieh H, Lu L, Kang C, Wu M, Lin C. Low-pressure pulsed focused ultrasound with microbubbles promotes an anticancer immunological response. *Journal of Translational Medicine.* 2012; 10(1):221.
- Lu P, Zhu X, Xu Z, Zhou Q, Zhang J, Wu F. Increased infiltration of activated tumor-infiltrating lymphocytes after high intensity focused ultrasound ablation of human breast cancer. *Surgery.* 2009; 145(3):286-293.
- Lynn JG, Zwemer RL, Chick AJ, Miller AE. A new method for the generation and use of focused ultrasound in experimental biology. *J Gen. Physiol.* 1942; 26:179-193.
- Katrina Chu F, Damian Dupuy E. Thermal ablation of tumours: biological mechanisms and advances in therapy, *Nature Reviews Cancer.* 2014; 14:199-208. doi:10.1038/nrc3672
- Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. *Nature Rev. Cancer.* 2013; 12:265-277.
- Robertson VJ, Baker KG. A review of therapeutic ultrasound: Effectiveness studies. *Physical therapy.* 2001; 81(7):1339-50. PMID 11444997.
- Rosberger D, Coleman D, Silverman R, Woods S, Rondeau M, Cunningham-Rundles S. Immunomodulation in choroidal melanoma: reversal of inverted CD4/CD8 ratios following treatment with ultrasonic hyperthermia. *Biotechnol. Ther.* 1994; 5(1-2):59-68.

21. Tiong L, Maddern GJ. Systematic review and meta-analysis of survival and disease recurrence after radiofrequency ablation for hepatocellular carcinoma. *Br. J Surg.* 2011; 98:1210-1224.
22. Wang X, Sun J. High-intensity focused ultrasound in patients with latestage pancreatic carcinoma. *Chinese Medical Journal.* 2002; 115:1332-1335.
23. Wu F, Wang Z, Lu P, Xu Z, Chen W, Zhu H *et al.* Activated anti-tumor immunity in cancer patients after high intensity focused ultrasound ablation. *Ultrasound in Medicine & Biology.* 2004; 30(9):1217-1222.
24. Wu F. High intensity focused ultrasound ablation and antitumor immune response. *The Journal of the Acoustical Society of America.* 2013; 134(2):1695.
25. Xia J, Xie F, Ran L, Xie X, Fan Y, Wu F. High-Intensity Focused Ultrasound Tumor Ablation Activates Autologous Tumor-Specific Cytotoxic T Lymphocytes. *Ultrasound in Medicine & Biology.* 2012; 38(8):1363-1371.
26. Xing Y, Lu X, Pua EC, Zhong P. The effect of high intensity focused ultrasound treatment on metastases in a murine melanoma model. *Biochem. Biophys. Res. Commun.* 2008; 375:645-650.