FOCUSED ULTRASOUND FOUNDATION

Focused Ultrasound and Cancer Immunotherapy

Overview

A number of therapeutic modalities including radiation, radiofrequency and laser-induced heating, cryoablation, and focused ultrasound have been shown to stimulate an immune response which can be enhanced by immunotherapeutic drugs.[1-6]

Focused ultrasound (FUS) has certain attributes that create the potential for a unique role in cancer immunotherapy when compared to the other modalities. The effects of FUS are highly selective with very sharp margins between the treated tumor tissue and adjacent normal structures. FUS can provide uniform or conformal treatment of the entire tumor volume. Unlike radiation therapy, FUS produces immediate effects that are non-ionizing, and there is no theoretical limit on the volume of tissue that can be treated or on the number of retreatments. Because FUS is non-invasive, it is associated with less pain and discomfort and decreased risk of complications including infection, hemorrhage, and collateral tissue damage.[7]

Furthermore, focused ultrasound is unique among ablative modalities in that it has four distinct modes that can promote an immune response (See Figure 1). The first, thermal ablation, is similar to many other technologies in that the targeted tissue is heated, causing coagulative necrosis. The second mode is mechanical cell lysis via histotripsy, a non-thermal process which produces shock waves that disrupt the tissue. Both thermal and mechanical ablation create and release neoantigens from damaged cancer cells, initiating a powerful anti-tumoral immune response. The third mode is prolonged moderate hyperthermia, which triggers the release of inflammatory heat shock proteins and an influx of immune cells.[14,15] In addition to these three modes, FUS can be used in combination with microbubbles to enhance the effectiveness of immunotherapeutics and anti-tumor immune cells by increasing vascular permeability and disrupting the tumor stroma to permit increased drug and immune cell trafficking within the tumor.[16,18]

Several preclinical and clinical studies have demonstrated that FUS can elicit an immune response (see Table 1). In a study comparing FUS treatment before radical mastectomy to surgery alone, patients who had received the focused ultrasound treatment prior to surgery had significantly higher tumor-infiltrating T cells, B cells, and NK cells.[2] In pancreatic tumors, FUS treatment increased the number of anti-tumor T and NK cells within the tumor.[23] Numerous other preclinical and clinical studies have shown that FUS treatment boosts various elements of the immune response through increases in cytotoxic T cell activity,[8,9] dendritic cells activation,10 heat shock protein and ATP up-regulation,[11,12] and circulating helper T cells lymphocytes.[13] In laboratory animals, FUS treatment has led to enhanced overall survival and protection from the growth of new tumors

While initial results seem promising, this is still early-stage research, particularly in assessing the impact of FUS on tumor growth, metastasis, and long-term survival.[10,19] Future work is needed to more fully understand the different mechanisms for FUS-induced immune response and how to most effectively use them in combination with immunotherapy.

Figure 1

Focused Ultrasound-Induced Immunomodulation

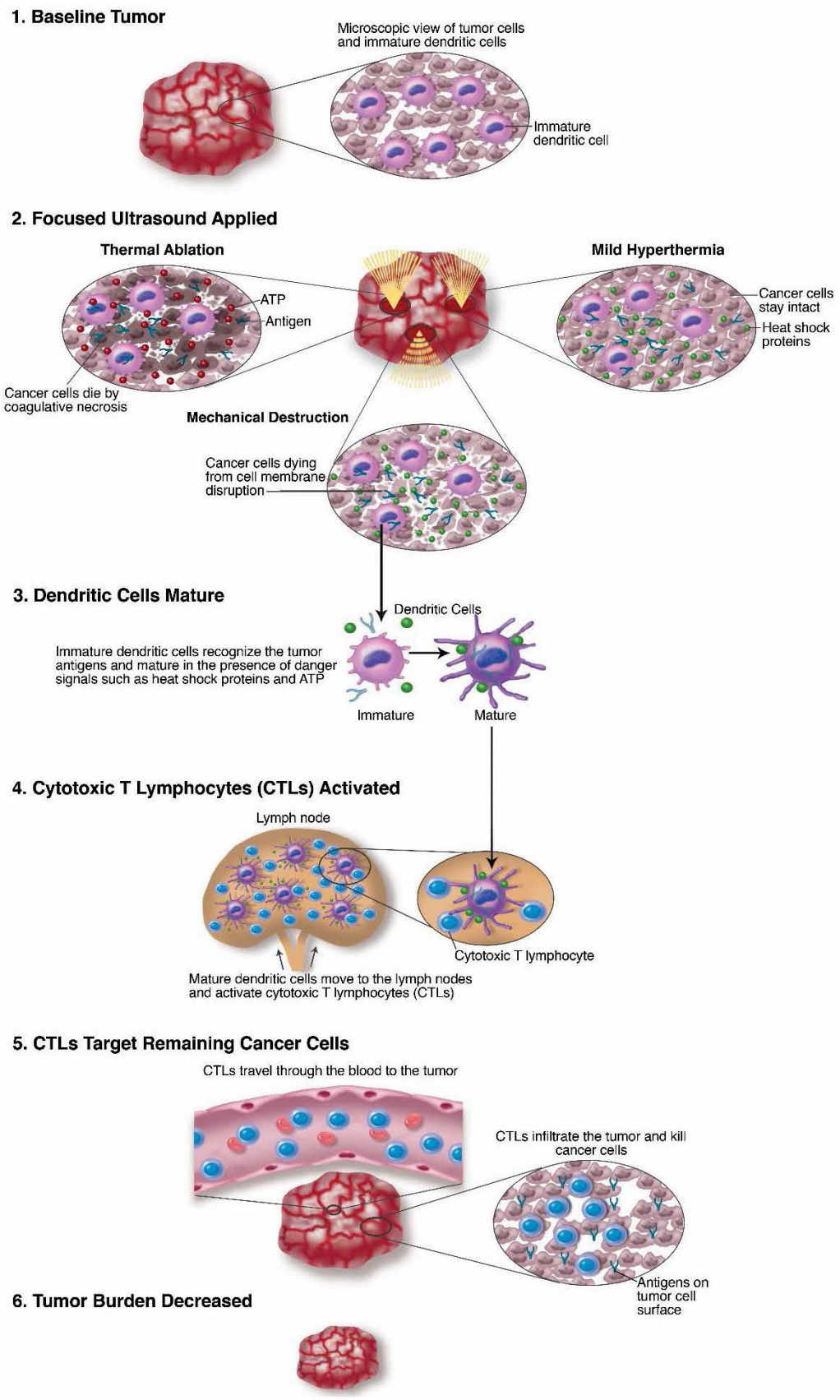

Table 1

Immunologic effects of focused ultrasound
Publications

Paper	Year	Clinical or Preclinical	Indication, Number of Patients	Immunologic Effect
Yang et al.[20]	1992	Preclinical	Neuroblastoma	Resistance to tumor re-challenge
Rosberger et al.[21]	1994	Clinical	Choroidal Melanoma (5)	2/3 patients reverted CD4+/CD8+ ratio from abnormal levels
Madersbacher et al.[22]	1998	Clinical	Prostate Cancer (5), Bladder Cancer (4)	Increase in HSP27 expression. Up-regulation strongest 2-3 hr after ablation, but still demonstrable after 5-8 days
Wang and Sun[23]	2002	Clinical	Pancreatic Cancer (15)	Increased CD3+ & CD4+ cells and CD4+/CD8+ ratio in 10 patients, not significant. NK cell activity was significantly enhanced
Kramer et al.[24]	2004	Clinical	Prostate Cancer (6)	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
Wu et al.[13]	2004	Clinical	Osteosarcoma (6), Hepatocellular Carcinoma (5), Renal Cell Carcinoma (5)	Increased CD4+ T cells and CD4+/CD8+ ratio
Hu et al.[11]	2005	Preclinical	Colon Adenocarcinoma	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
Hundt et al.[12]	2007	Preclinical	Melanoma, Fibroma, Squamous Cell Carcinoma	HSP70 expression can be induced at lower temperatures than heat stress alonetemperatures than
Wu et al.[25]	2007	Clinical	Breast Cancer (23)	Epithelial membrane antigen and HSP70 had 100% expression in tumor debris. Other proteins found were CA15-3 (52%), TGF-beta1 (57%), TGF-beta2 (70%), IL-6 (48%), IL-10 (61%), and VEGF (30%)
Zhou et al.[26]	2007	Preclinical	Hepatocellular Carcinoma	Increased CD4+ levels and CD4+/CD8+ ratio. Decreased CD8+ levels. Resistance to re-challenge
Zhou et al.[27]	2007	Clinical	Liver Cancer (13), Sarcoma (2)	Decreased serum VEGF, TGF-beta1, and TGF-beta2 levels. Patients without metastases had significantly lower immunosuppressive cytokine levels
Kruse et al.[28]	2008	Preclinical	[Transgenic Reporter Mice]	Peak HSP70 expression at 6-48 hours after treatment. Significant activity up to 96 hours after treatment

Table 1

Immunologic effects of focused ultrasound

Publications continued

Paper	Year	Clinical or Preclinical	Indication, Number of Patients	Immunologic Effect
Xing et al.[29]	2008	Preclinical	Melanoma	Increased CTL cytotoxicity. No increased risk of metastasis after HIFU treatment
Deng et al.[10]	2009	Preclinical	Hepatocellular Carcinoma	Increased CTL cytotoxicity, DC activation, IFN-gamma and TNF-alpha secretion
Xu et al.[30]	2009	Clinical	Breast Cancer (23)	Increase in local infiltration and activation of DCs and macrophages. Greater DC and macrophage expression of HLA-DR, CD80, and CD86 in the HIFU group
Lu et al.[31]	2009	Clinical	Breast Cancer (23)	Increased tumor-infiltrating B lymphocytes, NK cells, and CD3, CD4+, and CD8+ T cells. Increased CD4+/CD8+ ratio and FasL+, granzyme+, and perforin+ TILs
Liu et al.[32]	2010	Preclinical	Melanoma	Increased DC infiltration and maturation. Sparse-scan mode more effective than dense-scan at enhancing DC infiltration and maturation
Zhang et al.[19]	2010	Preclinical	Hepatocellular Carcinoma	Increased CTL cytotoxicity and DC activation. Resistance to tumor re-challenge
Xia et al.[9]	2012	Preclinical	Hepatocellular Carcinoma	Increased CTL cytotoxicity, IFN-gamma secretion, TNF-alpha secretion, and number of tumor-specific CTLs
Huang et al.[33]	2012	Preclinical	Prostate Cancer	Resistance to tumor re-challenge. Down-regulation of STAT3. Increased CTLs and decreased Tregs in the spleen and tumor draining lymph nodes
Liu et al.[16]	2012	Preclinical	Colorectal Carcinoma	FUS + microbubbles increased TILs, infiltration of CTLs, and the CD8+/Treg ratio
Alkins et al.[18]	2013	Preclinical	Metastatic Brain Tumor	FUS + microbubbles enables NK cells to cross the blood-brain barrier to target brain tumors
Kheirolomoom et al.	2015	Preclinical	Breast Cancer	FUS + CpG + CuDox increased leukocytes, CD4+ and CD8+ T-effector cells and reduced myeloid-derived suppressor cells.
Hunt et al.	2015	Preclinical	Melanoma	Low power FUS caused tumor hypoxia and increased T cell infiltration.
Chen et al.	2015	Preclinical	Brain	FUS-induced immune response during BBB opening.

References

1. Drake, C. G. Combination immunotherapy approaches. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. ESMO* **23 Suppl 8**, viii41–46 (2012).
2. Higgins, J. P., Bernstein, M. B. & Hodge, J. W. Enhancing immune responses to tumor-associated antigens. *Cancer Biol. Ther.* **8**, 1440–1449 (2009).
3. Isbert, C. et al. Enhancement of the immune response to residual intrahepatic tumor tissue by laser-induced thermotherapy (LITT) compared to hepatic resection. *Lasers Surg. Med.* **35**, 284–292 (2004).
4. Wu, F. High intensity focused ultrasound ablation and antitumor immune response. *J. Acoust. Soc. Am.* **134**, 1695–1701 (2013).
5. Gulley, J. L. et al. Combining a Recombinant Cancer Vaccine with Standard Definitive Radiotherapy in Patients with Localized Prostate Cancer. *Clin. Cancer Res.* **11**, 3353–3362 (2005).
6. Chi, K.-H. et al. Combination of conformal radiotherapy and intratumoral injection of adoptive dendritic cell immunotherapy in refractory hepatoma. J. Immunother. *Hagerstown Md 1997* **28**, 129–135 (2005).
7. Kim, Y., Rhim, H., Choi, M. J., Lim, H. K. & Choi, D. High-Intensity Focused Ultrasound Therapy: an Overview for Radiologists. *Korean J. Radiol.* **9**, 291–302 (2008).
8. Hu, Z. et al. Investigation of HIFU-induced anti-tumor immunity in a murine tumor model. *J. Transl. Med.* **5**, 34 (2007).
9. Xia, J.-Z. et al. High-intensity focused ultrasound tumor ablation activates autologous tumor-specific cytotoxic T lymphocytes. *Ultrasound Med. Biol.* **38**, 1363–1371 (2012).
10. Deng, J., Zhang, Y., Feng, J. & Wu, F. Dendritic cells loaded with ultrasound-ablated tumour induce in vivo specific antitumour immune responses. *Ultrasound Med. Biol.* **36**, 441–448 (2010).
11. Hu, Z. et al. Release of endogenous danger signals from HIFU-treated tumor cells and their stimulatory effects on APCs. Biochem. *Biophys. Res. Commun.* **335**, 124–131 (2005).
12. Hundt, W., O'Connell-Rodwell, C. E., Bednarski, M. D., 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.* **14**, 859–870 (2007).
13. Wu, F. et al. Activated anti-tumor immunity in cancer patients after high intensity focused ultrasound ablation. *Ultrasound Med. Biol.* **30**, 1217–1222 (2004).
14. Skitzki, J. J., Repasky, E. A. & Evans, S. S. Hyperthermia as an immunotherapy strategy for cancer. *Curr. Opin. Investig. Drugs Lond. Engl. 2000* **10**, 550–558 (2009).
15. Partanen, A. et al. Mild hyperthermia with magnetic resonance-guided high-intensity focused ultrasound for applications in drug delivery. *Int. J. Hyperthermia* **28**, 320–336 (2012).
16. Liu, H.-L. et al. Low-pressure pulsed focused ultrasound with microbubbles promotes an anticancer immunological response. *J. Transl. Med.* **10**, 221 (2012).
17. Unga, J. & Hashida, M. Ultrasound induced cancer immunotherapy. *Adv. Drug Deliv. Rev.* **72**, 144–153 (2014).
18. Alkins, R. et al. Focused ultrasound delivers targeted immune cells to metastatic brain tumors. *Cancer Res.* **73**, 1892–1899 (2013).
19. Zhang, Y., Deng, J., Feng, J. & Wu, F. Enhancement of antitumor vaccine in ablated hepatocellular carcinoma by high-intensity focused ultrasound. *World J. Gastroenterol. WJG* **16**, 3584–3591 (2010).
20. Yang, R. et al. Effects of high-intensity focused ultrasound in the treatment of experimental neuroblastoma. *J. Pediatr. Surg.* **27**, 246–250; discussion 250–251 (1992).
21. Rosberger, D. F. et al. Immunomodulation in choroidal melanoma: reversal of inverted CD4/CD8 ratios following treatment with ultrasonic hyperthermia. *Biotechnol. Ther.* **5**, 59–68 (1994).
22. Madersbacher, S. et al. Regulation of heat shock protein 27 expression of prostatic cells in response to heat treatment. *The Prostate* **37**, 174–181 (1998).
23. Wang, X. & Sun, J. High-intensity focused ultrasound in patients with late-stage pancreatic carcinoma. *Chin. Med. J. (Engl.)* **115**, 1332–1335 (2002).
24. Kramer, G. et al. Response to sublethal heat treatment of prostatic tumor cells and of prostatic tumor infiltrating T-cells. *The Prostate* **58**, 109–120 (2004).

References

25. Wu, F. et al. Expression of tumor antigens and heat-shock protein 70 in breast cancer cells after high-intensity focused ultrasound ablation. *Ann. Surg. Oncol.* **14**, 1237–1242 (2007).
26. Zhou, P., Fu, M., Bai, J., Wang, Z. & Wu, F. Immune response after high-intensity focused ultrasound ablation for H22 tumor. *ASCO Meet. Abstr.* **25**, 21169 (2007).
27. Zhou, Q. et al. Changes in circulating immunosuppressive cytokine levels of cancer patients after high intensity focused ultrasound treatment. *Ultrasound Med. Biol.* **34**, 81–87 (2008).
28. Kruse, D. E., Mackanos, M. A., O'Connell-Rodwell, C. E., Contag, C. H. & Ferrara, K. W. Short-duration-focused ultrasound stimulation of Hsp70 expression in vivo. *Phys. Med. Biol.* **53**, 3641–3660 (2008).
29. Xing, Y., Lu, X., Pua, E. C. & Zhong, P. The effect of high intensity focused ultrasound treatment on metastases in a murine melanoma model. *Biochem. Biophys. Res. Commun.* **375**, 645–650 (2008).
30. Xu, Z.-L. et al. Activation of tumor-infiltrating antigen presenting cells by high intensity focused ultrasound ablation of human breast cancer. *Ultrasound Med. Biol.* **35**, 50–57 (2009).
31. Lu, P. et al. Increased infiltration of activated tumor-infiltrating lymphocytes after high intensity focused ultrasound ablation of human breast cancer. *Surgery* **145**, 286–293 (2009).
32. Liu, F. et al. Boosting high-intensity focused ultrasound-induced anti-tumor immunity using a sparse-scan strategy that can more effectively promote dendritic cell maturation. *J. Transl. Med.* **8**, 7 (2010).
33. Huang, X. et al. M-HIFU inhibits tumor growth, suppresses STAT3 activity and enhances tumor specific immunity in a transplant tumor model of prostate cancer. *PloS One* **7**, e41632 (2012).