Release of tumor antigens from breast cancer cells by a novel ultrasound ablation method

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HISTOTRIPSY

Abstract

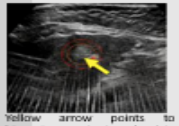
Background: HER2-positive breast cancer accounts for about 20% of all breast cancer cases and is one of the most aggressive subtypes of breast cancer (1,2). These patients are often resistant to current antitumor treatments including immunotherapy. Histotripsy (HT) is a non-invasive, non-thermal, non-ionizing ultrasound ablation method that utilizes high-pressure, microsecond ultrasound pulses at low-duty cycle to create cavitation at a focal point (3). It has been reported that HT can not only effectively inhibit the tumor at its target site but also arrest tumor growth at the remote site (the abscopal effect) (3,4). The molecular mechanism of the abscopal effect remains unclear but is thought to be related to HT-triggered systemic anti-tumor immune responses. The release of immunogenic tumor antigens is critical in the induction of systemic and specific anti-tumor immune responses.
Methods: HER2-positive murine mammary tumor cells were injected directly into HER2 transgenic mouse's mammary fat pads or the subcutaneous flank. Tumor-bearing mice were treated with histotripsy with acute tumor resection. Treated tumors were respected and separated into cell-free and residual cell pellet fractions using centrifugation. Total protein concentration in both fractions was quantified using BCA protein assays. Ultra-performance liquid chromatography (UPLC) and Western blotting were used to evaluate and characterize HER2 protein. Histotripsy was performed using a custom built 8-element, 1 MHz transducer with ultrasound-guided targeting. The tested treatment dose was defined as the number of pulse repetitions per focal location (ppl). Then, 100 ppl was chosen for this study. Other acoustic parameters such as pulse repetition frequency remained fixed at 100 Hz PRF.
Results: Our results demonstrated that the HT cavitation can accurately target and disrupt tumor tissues and significantly release more total proteins into the cell free fraction than untreated tumor by BCA assay. HT treatment significantly increases the level of released HER2 protein as measured by UPLC and Western blot analysis. UPLC analysis further showed that the cell-free fractions exhibited a protein peak with a retention time at around 9.3 minutes. This released antigen appeared identical to recombinant HER2 protein, suggesting that the HER2 released by HT is in line with intact protein.
Conclusions: Our findings have demonstrated that HT-treated orthotopic breast tumor could trigger the release of intact tumor antigens, which in some manner can stimulate the host immune system. The release of cell-free tumor antigens can not only create the basis for HT-induced systemic anti-tumor immune responses, but also a favorable environment for immunotherapies. Finally, this increase in immune activity potential enhances the host's ability to prevent tumor recurrence and metastasis.

Introduction

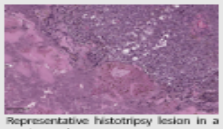
Histotripsy is a therapeutic modality which uses short (<20 µs), intense (|p-|>15 MPa) ultrasound pulses with <1% duty cycle to mechanically fractionate tissue through cavitation and was FDA approved in 2023 for the treatment of human liver tumors (3).

Histotripsy treatment of primary tumors can induce a systemic anti-tumor immune response strong enough to reduce tumor burden. This is believed to be partially due to the release of tumor antigens recognizable to the immune system.

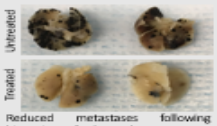
In this study we are interested in detecting and quantifying the release of tumor antigen in a clinically relevant HER2-positive breast cancer model (6).



Yellow arrow points to histotripsy cavitation cloud in a human liver (5).



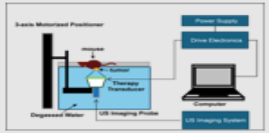
Representative histotripsy lesion in a murine melanoma tumor



Reduced metastases following histotripsy of subQ melanoma tumor

Materials and Methods

A transgenic C57BL/6 mouse expressing the human HER2 and EO771E2 cells were used for this study. Histotripsy was performed using an 8-element 1 MHz for the subcutaneous tumor treatments and a 1.1 MHz transducer for the orthotopic tumor treatments.

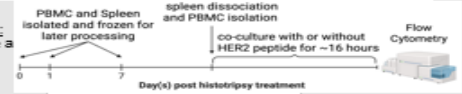




Experiment 1: Immediate HER2 antigen after subcutaneous treatment



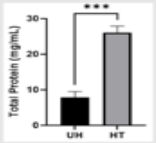
Experiment 2: Antigen co-culture a orthotopic Treatment



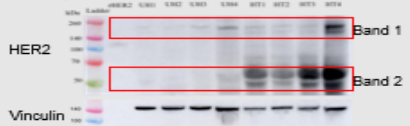
Tumor antigen release

In vivo histotripsy (HT) of EO771E2 tumors results in an overall increase in total cell-free peptide compared to untreated (UH) tumor samples (Figure 1A). This increase in overall peptide correlates with a significant increase in the amount of detected cell-free HER2 with both western blot (Figure 2B) and UPLC (Figure 2C).

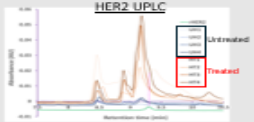
A) Total cell-free peptide



B) HER2 Western Blot

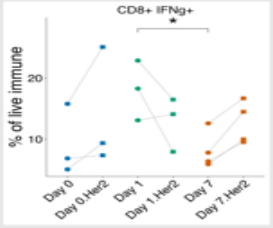


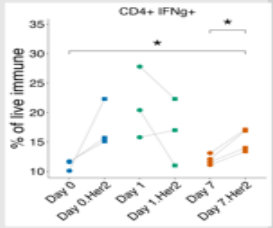
C) HER2 UPLC



PBMC co-culture with HER2 antigen

At 7 days post-treatment, the percentages of both IFN-γ CD4+ T cells and CD8+ T cells were significantly increased in peripheral blood mononuclear cells (PBMC) exposed to the HER2 antigen compared to PBMC not exposed to it. This suggested HT treatment could lead to induce antigen specific cellular response.





Discussion

Understanding the patient's immune microenvironment is crucial for implementing immunotherapy (7). Here we demonstrate the feasibility of detecting changes in IFN-γ expression in circulating antigen-specific T cells in a HER2-positive breast cancer mouse model treated with Histotripsy. This is a non-invasive biomarker that may hold promise for predicting the potential for patients to develop an anti-tumor immune response after the treatment. Furthermore, understanding the kinetics of antigen sensitivity is an important step in guiding rational combination therapy regimens with Histotripsy

Conclusions

- In this study we show that orthotopic and subcutaneous treatments of breast cancer tumors with histotripsy lead to the release of minimally degraded cell-free tumor antigens.
- The release of tumor antigens correlates with early changes in IFN-γ expression in cognate circulating antigen-specific T cells after antigen stimulation.
- The next step is to study T cells in the lymph node as well as measure potential tumor rechallenge protection following histotripsy of HER2-positive breast cancer tumors.

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