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## Treatment of Osteosarcoma with Microwave Thermal Ablation Inducing Immunogenic Cell Death

### Abstract

**Background:** Microwave ablation (MWA) has been used as a classical hyperthermia ablation technique for many decades, with the intention to induce direct tumor cell killing or modulation of tumor architecture. To protect the adjacent organs from excessive heating, the “gray zone” of ablation has been concerned to become a possible source of residual tumor or locoregional relapse.

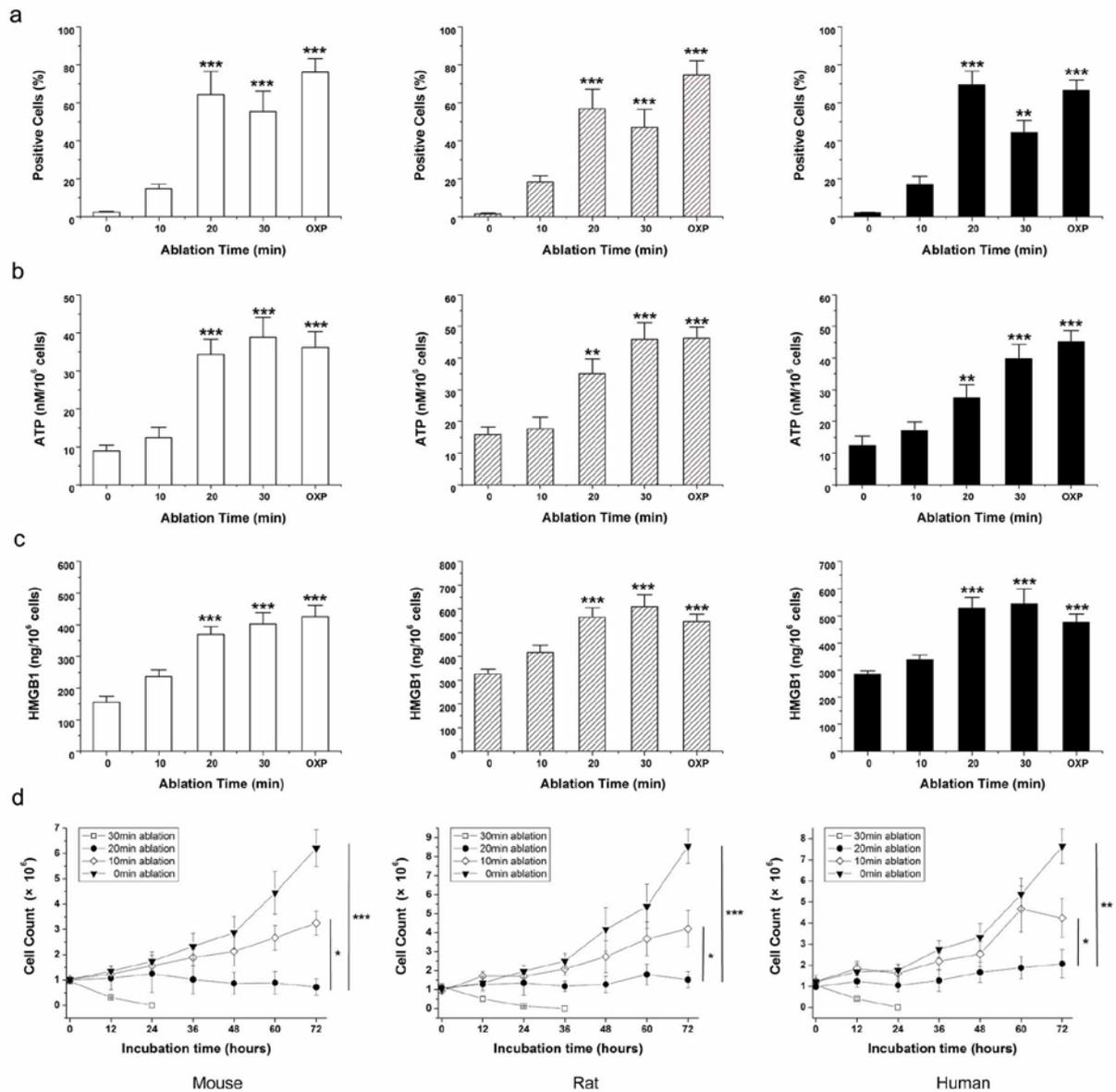
**Purposes:** The purpose of this study was to explore whether MWA induced tumor cell death could generate an immunogenic source of tumor antigens and elicit tumor-specific immune responses, taking an alternative antitumor effects.

**Methods:** Three kinds of osteosarcoma cell lines, respectively derived from mice, rats and human, were selected as ablation models. *In vitro* and *in situ* tumor ablation were both performed to detect the “damage-associated molecular patterns” (DAMPs) exposure level. Time-dependent molecular changes of ablated tumor tissue *in situ* were observed, and subsequent antitumor immune responses and potential mechanism were also explored.

**Results:** *In vitro* and *in situ* tumor ablation both induced time-dependent upregulation of DAMPs release and calreticulin (CRT) cell-surface expression. Active ablated products vaccination resulted in complete protection in both mouse and rat tumor-bearing models, which was mediated primarily by vaccine-elicited CD8<sup>+</sup> T cells. These effector cells functioned by releasing IFN- $\gamma$  and TNF- $\alpha$  in the presence of target cells, which may trigger FasL-directed cell apoptosis. *In situ* tumor ablation also demonstrated that high frequency of CD8<sup>+</sup> T cells were recruited into ablated tumor tissues, which was roughly correlated with the exposure level of CRT.

**Conclusion:** In the present study, we demonstrated that MWA processed osteosarcoma cells could be applied to generate specific antitumor effects, especially in *in situ* ablation strategy. This study offers a rational option for the use of microwave thermal ablation in combination with immunotherapy, especially for patients who have failed chemotherapy or have limited treatment options.

## Figures



**Figure 1. MWA induces a continuum of time-dependent cellular changes related to immunogenic cell death.**

Mouse osteosarcoma (K7M2, open bars), rat osteosarcoma (UMR106, hatched bars), and human osteosarcoma (MG63, black bars) cells were exposed to 10, 20 or 30 min MWA or mock irradiation at 0 min. Oxaliplatin (OXP; 1 mM/mL) was the positive control for ICD.

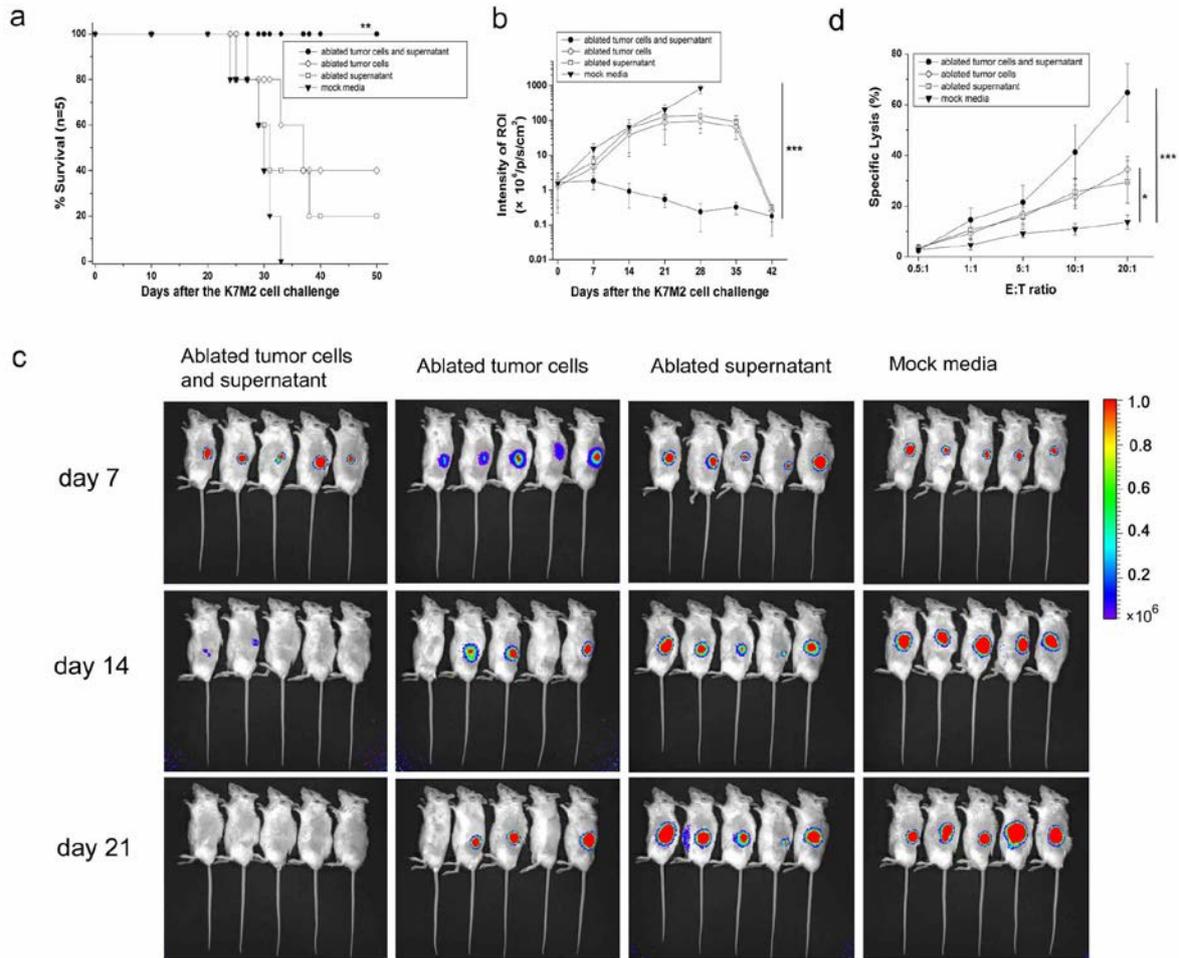
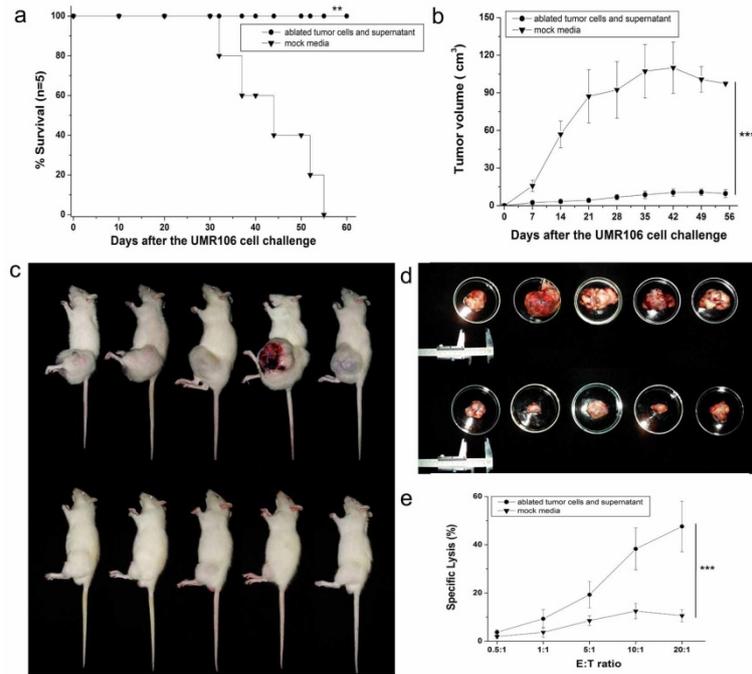
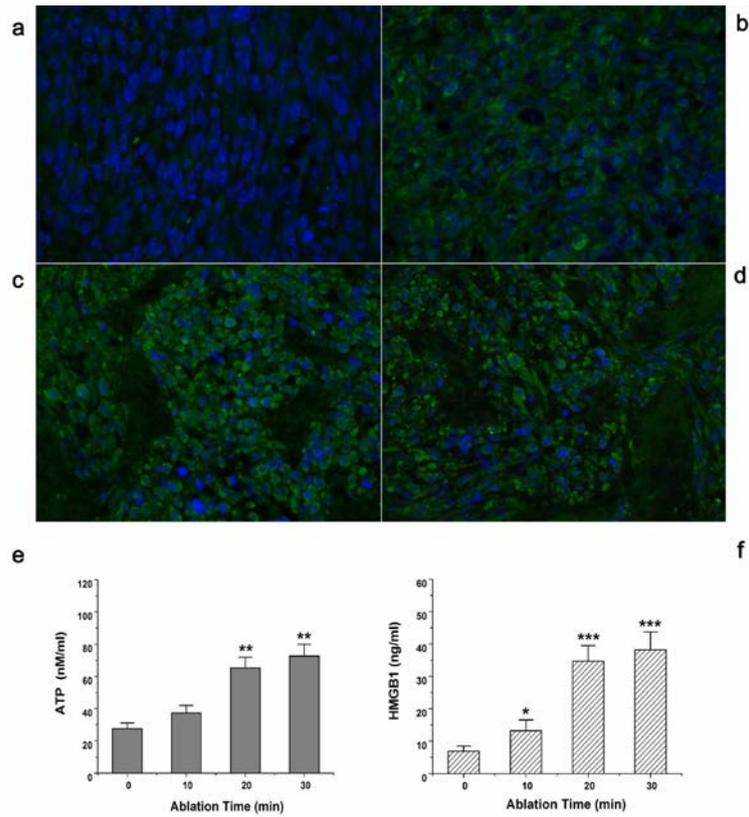


Figure 2. Complete protection of mice against lethal challenge with osteosarcoma cells.



**Figure 3. Complete protection of rats against lethal challenge with osteosarcoma cells.**



**Figure 4. Exposure of tumors to MWA in situ increased expression of CRT and release of ATP and HMGB1.**

SD rats bearing UMR106 transplanted tumors were exposed to time-dependent MWA (10, 20, 30 min) or left untreated (0 min). After 24 h, tumors were removed surgically, and evaluated by immunofluorescence staining (40×) for CRT expression.

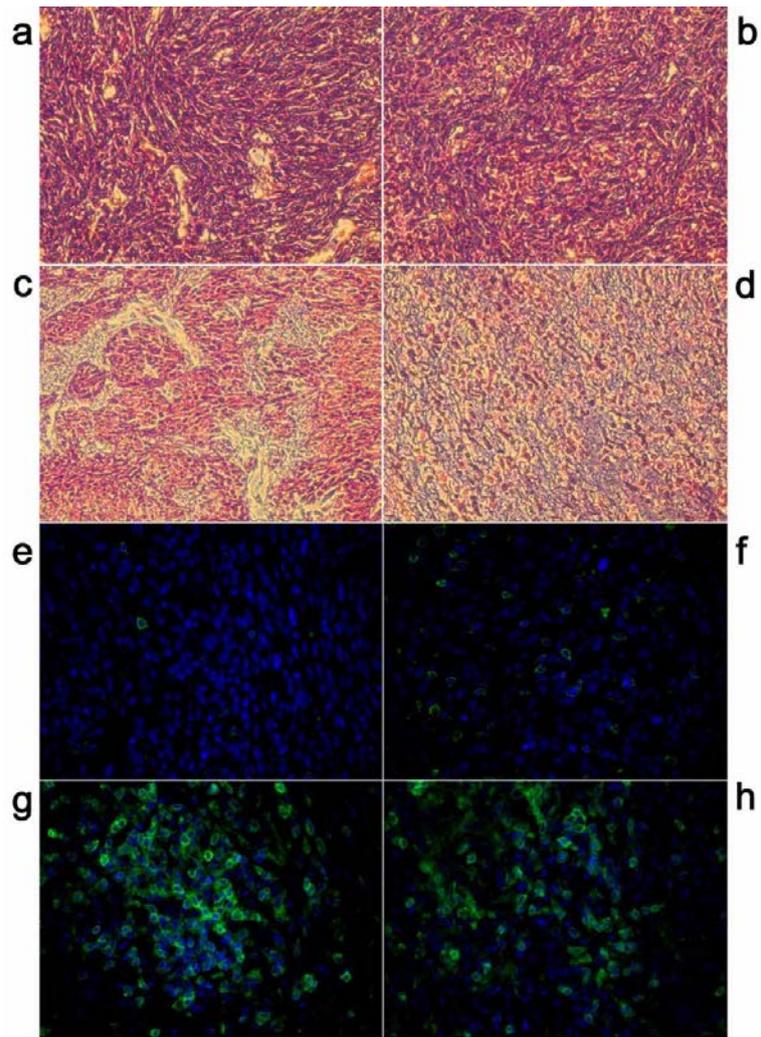


Figure 5. Exposure of tumors to MWA in situ showed histologic changes and increases in CD8 population in rat osteosarcoma tissue.

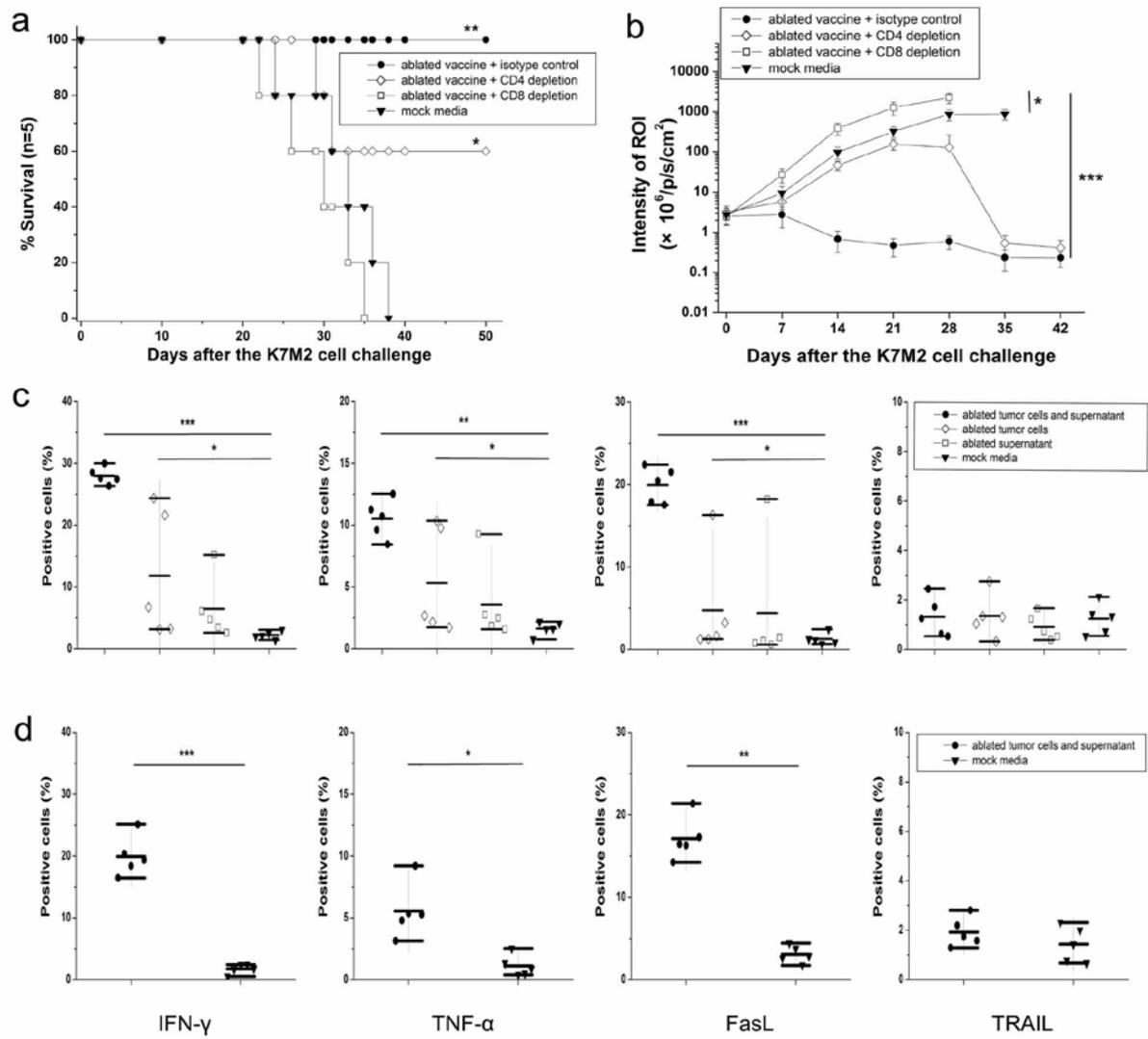


Figure 6. Mechanism of killing of osteosarcoma cells induced by vaccine-elicited CD8+ T cells.