

Repeated treatment with modulated electro-hyperthermia inhibits tumor growth in a triple negative mouse breast cancer model

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Introduction: The effective therapy of triple-negative breast cancer (TNBC) has not yet been achieved. Modulated electro-hyperthermia (mEHT) is a novel adjuvant antitumor therapy, based on selective tumor cell killing by a 13.56 MHz radiofrequency induced electric field.

Aims: Our aim was to investigate the effects of repeated mEHT treatment in a TNBC bearing mouse model.

Method: 4T07 cells were inoculated orthotopically in female BALB/c mice. Tumor growth was monitored in vivo by digital caliper and ultrasound (Phillips Sonos 5500). The mEHT (n=8) or sham (n=9) treatments started 7 days after inoculation and were repeated 5 times, on every other day. Mice were treated 30 minutes long with 1.0 ± 0.5 W power to achieve 40°C skin temperature above the tumor. Mice were euthanized 1 day after the fifth treatment and the tumors were dissected, weighed and processed for histology and molecular biology techniques. The ratio of the damaged area compared to the whole tumor area (Tissue Destruction Ratio, TDR) was evaluated on H&E and cleaved caspase-3 stained sections, while HSP70, a common damage-associated molecular signal, Ki67, a proliferation marker and p21, a tumor suppressor protein expression were analyzed on immunohistochemical staining with the HistoQuant module of the CaseViewer Software (3DHistech).

Results: There was a significant decrease in tumor growth (sham: 5.7x, mEHT: 2.4x relative to pre-treatment (day 6) size, $p < 0.0001$) and weight (sham: 288.3 ± 58.1 mg vs mEHT: 85.3 ± 21.3 mg, $p < 0.05$) in the mEHT treated group, compared to the sham group. The HSP70 stained area in the non-destroyed tumor tissue was 5.2 fold higher in the mEHT treated group, compared to the sham group ($p < 0.05$). Moreover, the Ki67 positive nucleus / mm² count was significantly lower (sham: 2823.4 ± 211.9 pcs/mm² vs mEHT: 1736.7 ± 315.3 pcs/mm², $p < 0.05$) and the p21 positive nucleus / mm² count showed increasing tendency (sham: 127.0 ± 25.3 pcs/mm² vs mEHT: 242.2 ± 78.2 pcs/mm², $p = 0.073$) in the mEHT treated group, compared to the sham group.

Conclusion: Our findings suggest, that repeated modulated electro-hyperthermia could inhibit tumor cell proliferation by promoting cell cycle arrest in vivo. Thus, mEHT could be a possible alternative adjuvant therapeutic strategy for TNBC cancer patients.

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oral presentation

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Introduction

Triple negative breast cancer

- 15% of breast cancer
- Highly aggressive
- High mortality
- Poor clinical outcome
- No targeted therapy



Modulated electro-hyperthermia (mEHT)

- Non-invasive therapeutic option
- Electromagnetic field generated by amplitude modulated 13.56 MHz radiofrequency

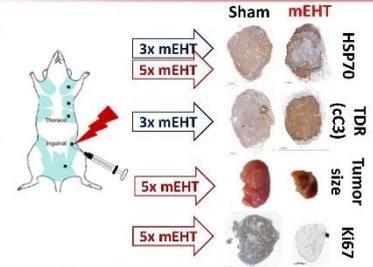
Discussion

3x repeated mEHT treatment induced

- Heat shock response
- Considerable tissue destruction
- Inhibition of tumor growth (4T1)

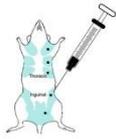
5x repeated mEHT treatment induced

- Heat shock response
- Decrease of cell proliferation
- Inhibition of tumor growth (4T07)



Methods

Inoculation



10⁶ 4T1 or 4T07 cells in 50ul PBS
 ♀ Balb/c mice
 4th mammary gland

Treatments



LAB-EHY 200
 Pole electrode

Measurements



Ultrasound
 Digital caliper

Immunohistochemistry



Hsp70
 cC3

Experimental protocol with 3 treatments

Day after inoculation	0	6	7	8	9	10	11	12
4T07 cell inoculation	x							
Tumor size (US, caliper)		x		X		x		x
mEHT			x		x		x	
Harvest								x

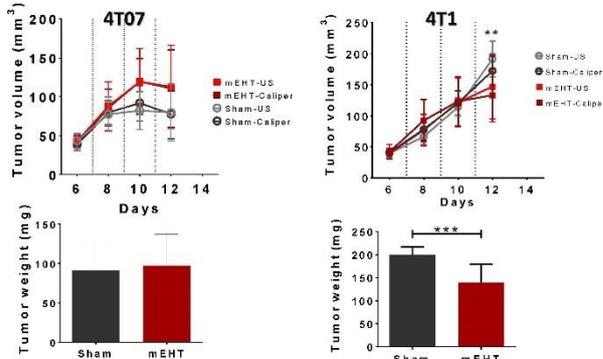
Experimental protocol with 5 treatments

Day after inoculation	0	6	7	8	9	10	11	12	13	14	15	16
4T07 cell inoculation	x											
Tumor size (US, caliper)		x		x		x		x		x		x
mEHT			x		x		x		x		x	
Harvest												x

Results

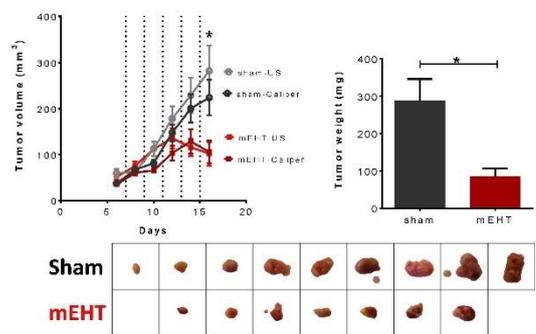
Three treatments

Tumor size changes after 3 mEHT treatments



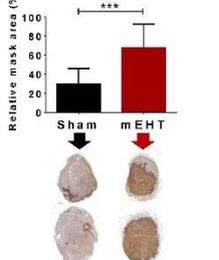
Five treatments

Tumor size changes after 5 mEHT treatments

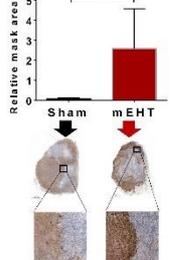


Molecular changes after 3 mEHT treatments

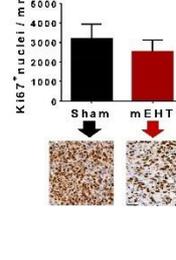
Tumor Destruction Ratio (cc3 expression)



Hsp70 expression

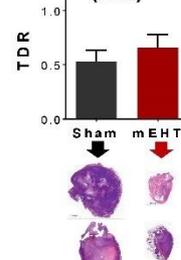


Ki67 expression

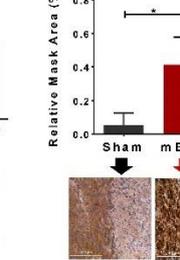


Molecular changes after 5 mEHT treatments

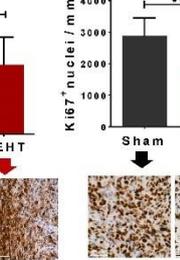
Tumor Destruction Ratio (H&E)



Hsp70 expression



Ki67 expression



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