Molecular mechanisms of modulated electrohyperthermia (mEHT) induced tumor damage

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Molecular mechanisms of modulated electrohyperthermia (mEHT) induced tumor damage

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Abstract

Modulated electro-hyperthermia (mEHT), a non-invasive, loco-regional complementary of radio- or chemotherapy, can by itself induce selective heat shock and cell stress in malignant tumors at ~42oC. Based on the published results we briefly summarize what has been revealed on the molecular background of tumor damage caused by mEHT treatment.

A single mEHT shot of 30-60 min provoked significant upregulation of γ-H2Ax (indicating DNA double strand brakes) and tumor destruction in colorectal cancer models, both in vitro and in vivo, dominantly following programmed tumor cell death mechanisms. Apoptotic response was diverse based on the (epi) genetic makeup of treated tumors and following both extrinsic (casp-8+) and intrinsic (translocated Bax & Cytochome C) caspase-dependent (casp-3+; in C26), or AIF-mediated (in p53 mutant HT29) caspase-independent pathways. Treatment response in C26 in vitro involved the upregulation of Ser15 phospho-p53 (indicating escape from Mdm2 control) and p21waf1 (the mediator of cell senescence), accompanied by the elevation of the pro-apoptotic PUMA, Bax and Bak-1 and the downregulation of the antiapoptotic XIAP, Bcl-2 and Bclx. Furthermore, mEHT treatment synergized with Doxorubicine chemotherapy. In histiocytic lymphoma (U937) both extrinsic and intrinsic caspase-dependent apoptosis was driven by phosphorylation of the c-Jun N-terminal kinases (JNK).

In vivo, early apoptosis was supplemented by complete cell cycle arrest shown by Ki67 negativity, and the occurrence and release of DAMP (damage associated molecular pattern) signals including chaperons such as calreticulin, Hsp70 and Hsp90 and the high mobility group1 (HMGB1) protein. After single treatment, the progressive tumor damage and accumulation of CD3 positive T-cells, including granzyme B+/CD8+ cytotoxic cells (granzyme B+/CD8- NK cells) as well as S100+ antigen presenting dendritic cells (APC), were consistent with a secondary, immunogenic cell death (ICD) mechanism added to the primary effect of mEHT. Furthermore, treatment response could be associated with elevated levels of glycolytic enzymes in vivo, and with increased lactate production and reduced buffer capacity (and pH) in cultures. mEHT treatment also supported antitumor immune response when combined with tumor-specific, intratumoral dendritic cell delivery involving tumor sites distant from the treated focuses (Abscopal effect).

In summary, radio- or chemotherapy can be supported by the inherent antitumor effects of mEHT, which can induce diverse, tumor-specific apoptosis pathways and antitumor immune response too. Besides direct heat induction in the extracellular space due to elevated glycolysis (Warburg-effect) and ion-concentration in cancer, mEHT may also act directly on cell membrane rafts (where local electric loss/absorbtion peaks), which concentrate ion

channels and transmembrane receptors. These features may explain the higher efficiency of mEHT compared to traditional hyperthermia under the same temperature.

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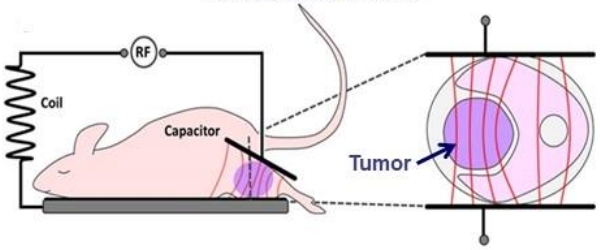
September 28-29, 2018

mEHT of 13.65 MHz – selective tumor targeting

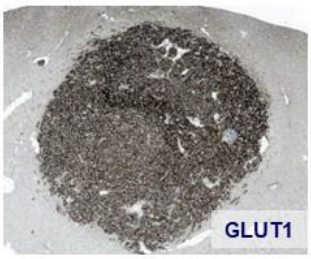
Enrichment of electric field in malignant tumors

- **Elevated:** glucose uptake, aerobe glycolysis (**Warburg-effect**)
  lactate$^-$ H^+ & other ion concentration & permittivity

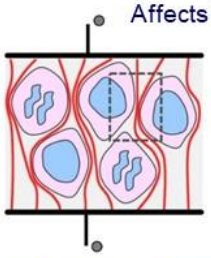
In vivo tumor model

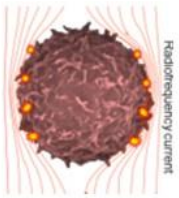


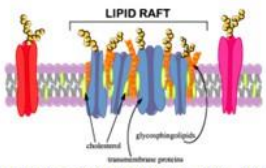
Lung cc liver metastasis



Affects EC and cell membrane





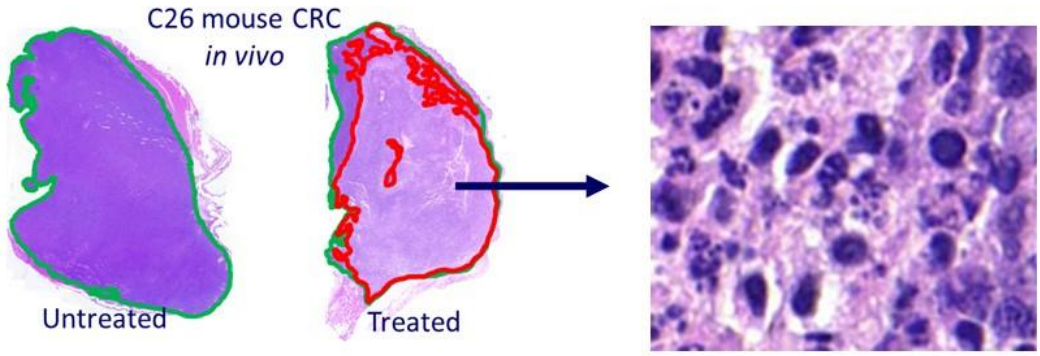


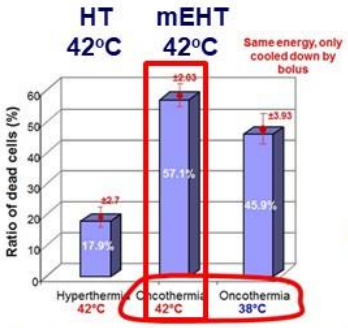
High dielectric potential in lipid rafts concentrating transmembrane receptors

- **Dielectric polarization/rotational friction – heat**

mEHT of 13.65 MHz – Significant tumor destruction

- **Mechanism: Programmed tumor cell death**





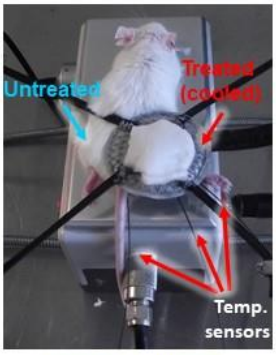
Synergy
Heat stress
+
Direct effect of EF

Additional effects compared to conventional heating

Andocs et al. Strahlenther Onkol. 2009,185:120-126.

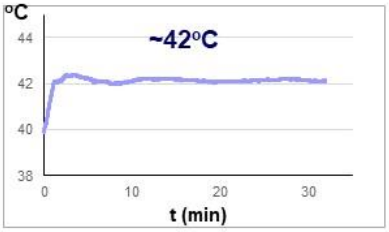
mEHT effects: *in vivo* & *in vitro* tumor models

Mouse: allo-, xenografts



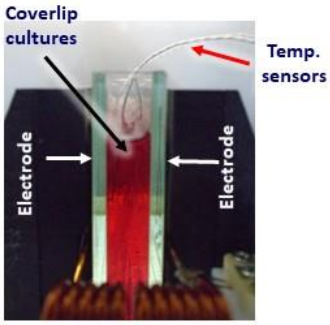
Symmetrical tumors
- **right leg - mEHT treated**
- **left leg - control**

Temperature control



mEHT: single/repeated 30 or 60 min
42 ±0.5°C (Lab EHY 100)

Tumor cell cultures



mEHT: mono-, or combined with chemo- or radio- or DC therapy

Tumor cell lines
- **Colorectal ADC: C26, C38 CRC, HT29,**
- **Lung ADC: LLT-H,**
- **Hepatoc. ADC: HepG2, Huh7**
- **Head-neck SCC: CCVII**
- **Glioma: U87-MG, A172**
- **Hist. lymphoma: U937**
- **Fibrosarcoma: FSall**

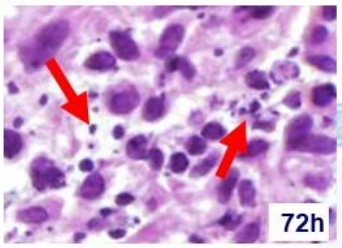
Published results from:
- Yonsei University College of Medicine Seoul, South Korea
- National University, Seoul, South Korea
- Tottori University, Japan
- Chiba University, Japan
- Toyama University, Japan
- Memorial Hospital, Taipei, Taiwan, ROC
- Chung Yuan Christian University,Taoyuan City, Taiwan, ROC
- Semmelweis University, Budapest, Hungary

mEHT induced programmed cell death (42°C)

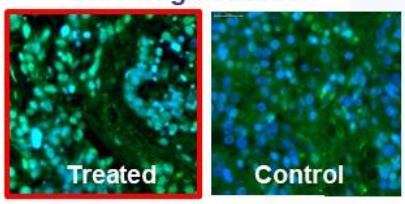
Colorectal cancer (CRC) cell lines: HT29 human (TP53 mutant); **C26 mouse**

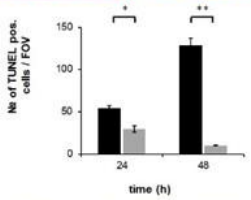
hHT29 CRC

Apoptotic bodies



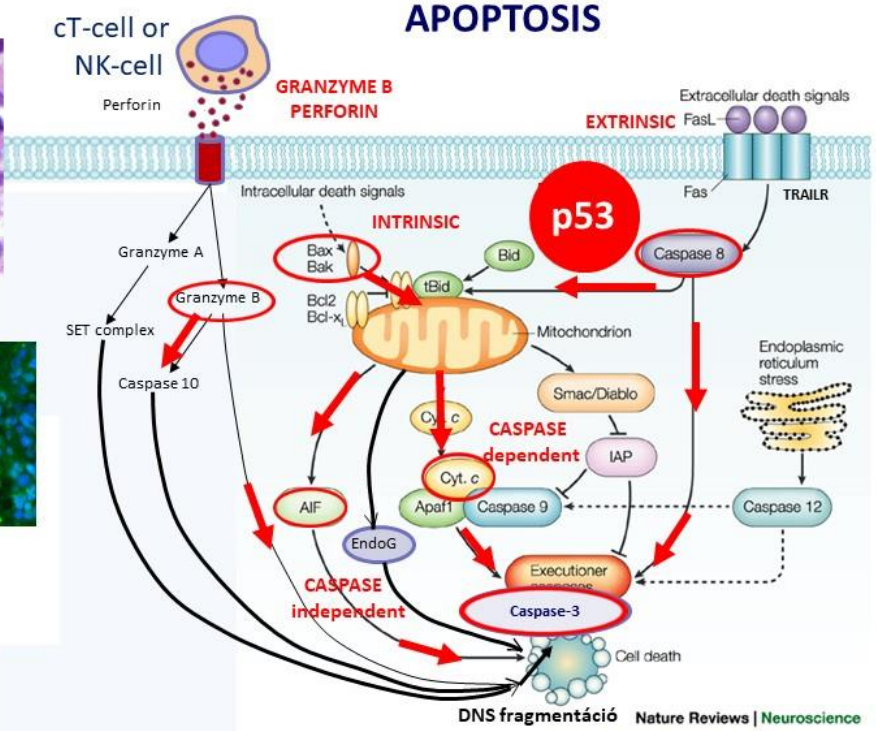
DNA fragmentation





Meggyeshazi, Andocs et al. Strahlenther Onkol. 2014, 190:815-822.

APOPTOSIS



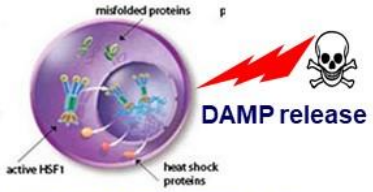
Vila-Przedborski: Apoptosis-Nature Reviews Neuroscience 2003, 4:365–375.

Early heat shock/cell stress & apoptosis response

hHT29 CRC

Upregulation of Hsp-s

HEAT SHOCK + CELL STRESS

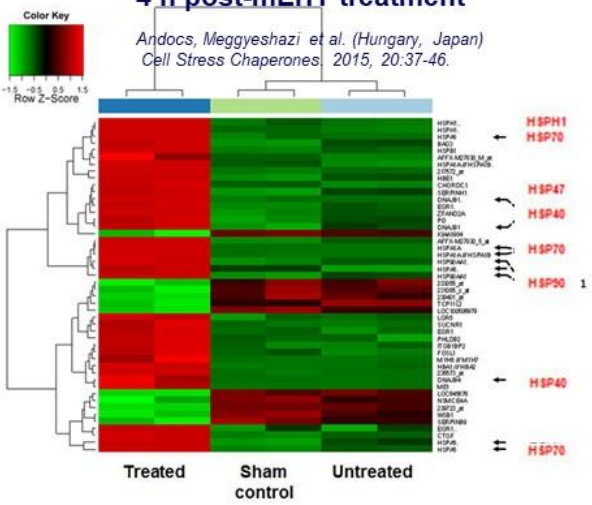


GeneChip human genom U133 Plus 2.0

6500 genes

4 h post-mEHT treatment

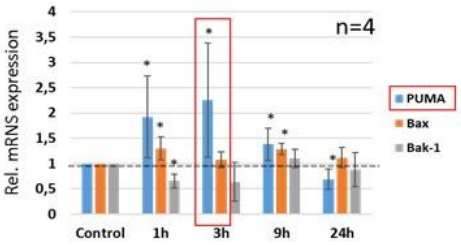
Andocs, Meggyeshazi et al. (Hungary, Japan) Cell Stress Chaperones. 2015, 20:37-46.



mC26 CRC

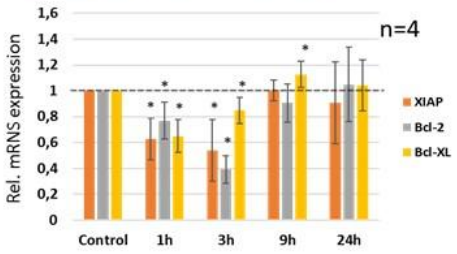
Pro-apoptotic (mRNA levels):

- **PUMA** (p53 upregulated modulator of apoptosis)
- **Bax** (Bcl-2-associated X)
- **Bak-1** (Bcl-2 homologous antagonist/killer)



Anti-apoptotic (mRNA levels):

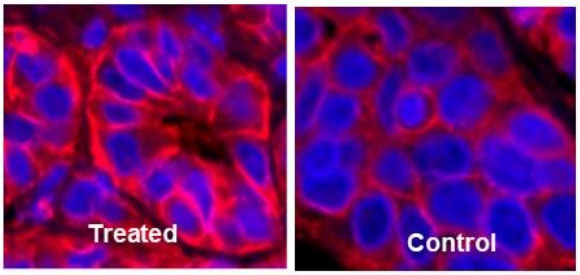
- **XIAP** (X-linked inhibitor of apoptosis)
- **Bcl-2** (B-cell lyphoma type-2 protein)
- **Bcl-XL** (B-cell lymphoma-extra large)

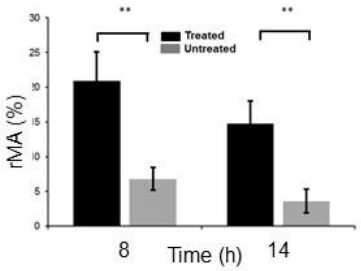


mEHT induced death receptor mediated extrinsic pathway

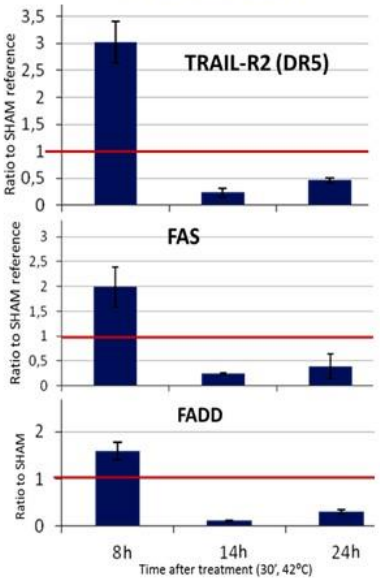
hHT29 (TP53 mutant) CRC xenograft

TRAIL-R2 (Death Receptor 5)





mRNA levels



Targeted therapy: **rh-Apo2L/TRAIL** and **MAbs** (*HGS ETR2/ lexatumumab*) - **agonist**

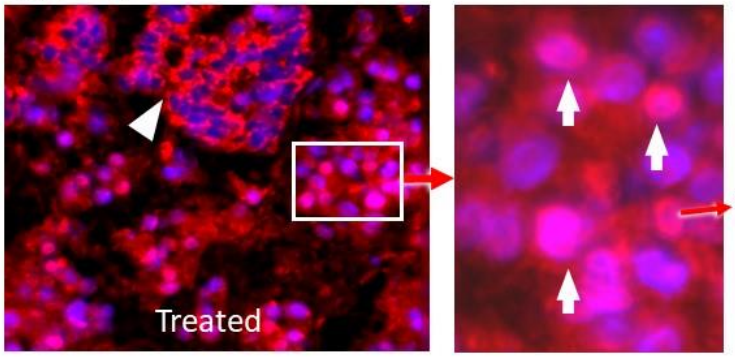
- **Suported cytotoxic chemo- and radiation therapy** in breast cancer & CRC
- **Phase-I-II** trials have been running

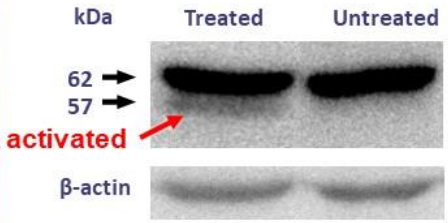
De Miguel et al. Cell Death and Differentiation 2016, 23:733–747

mEHT induced Caspase independent & dependent apoptosis

mHT29 CRC
(mTP53)

AIF (apoptosis inducing factor) **translocation (24h)**



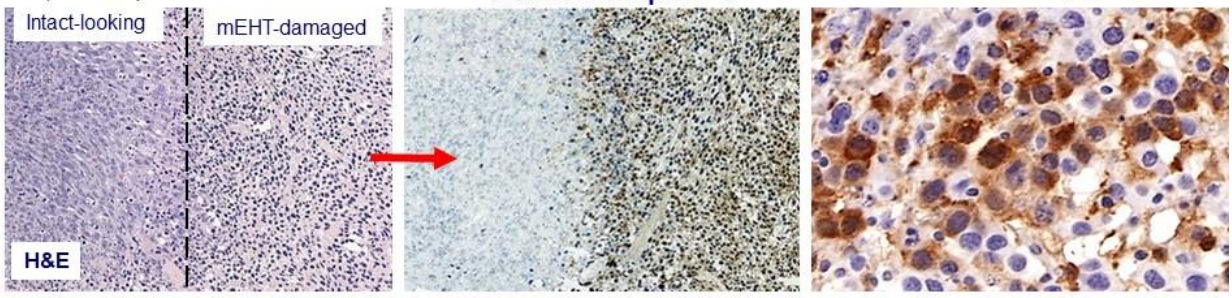


mC26 CRC
(wTP53)

Extrinsic Caspase dependent

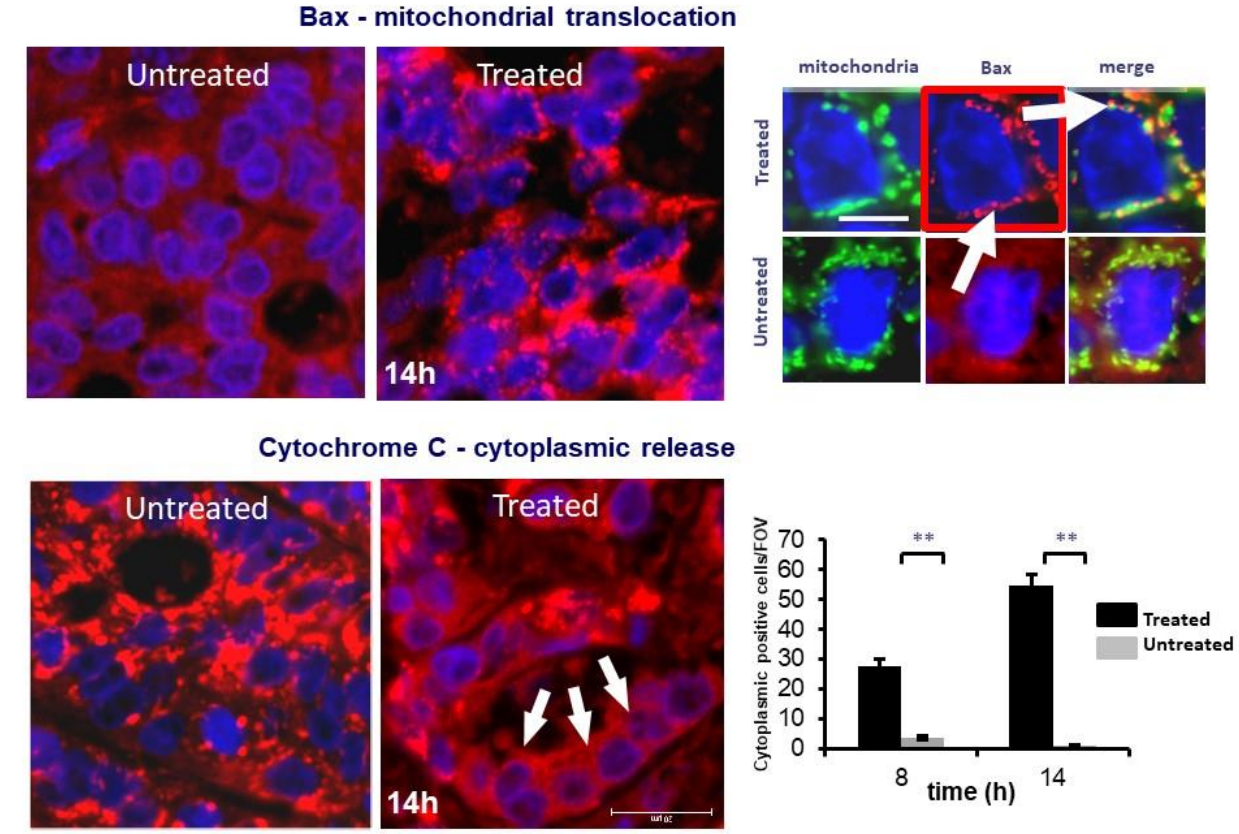
Cleaved-**Caspase 8**

Cleaved-**Caspase 3**

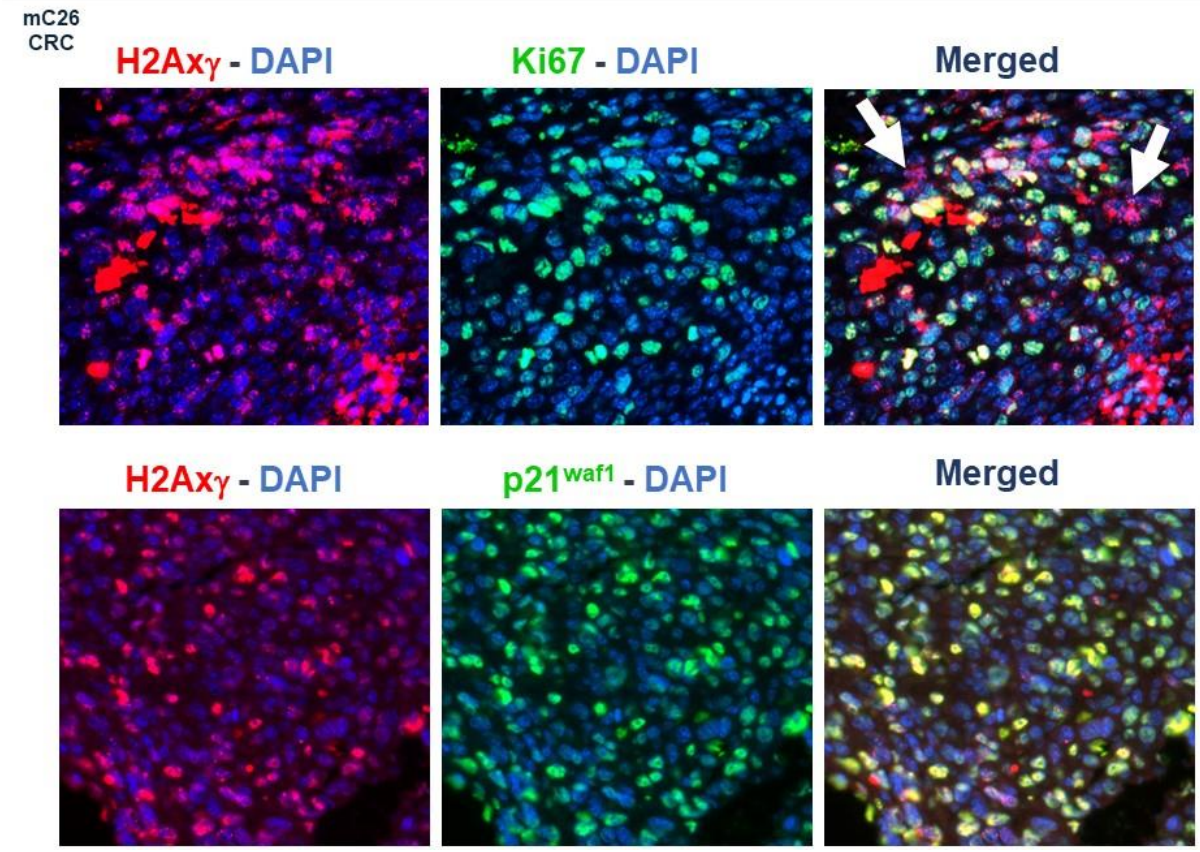


24h post-treatment

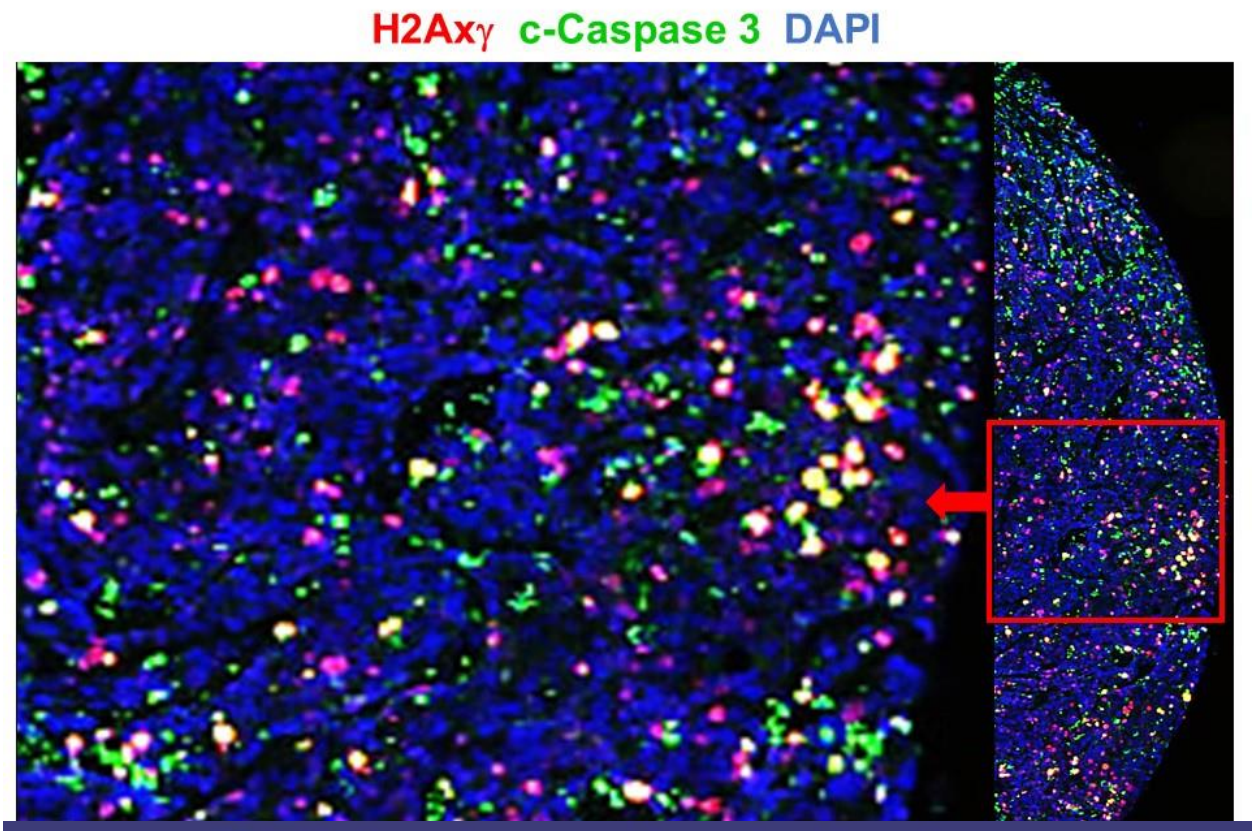
mEHT induced Caspase-dependent intrinsic pathway



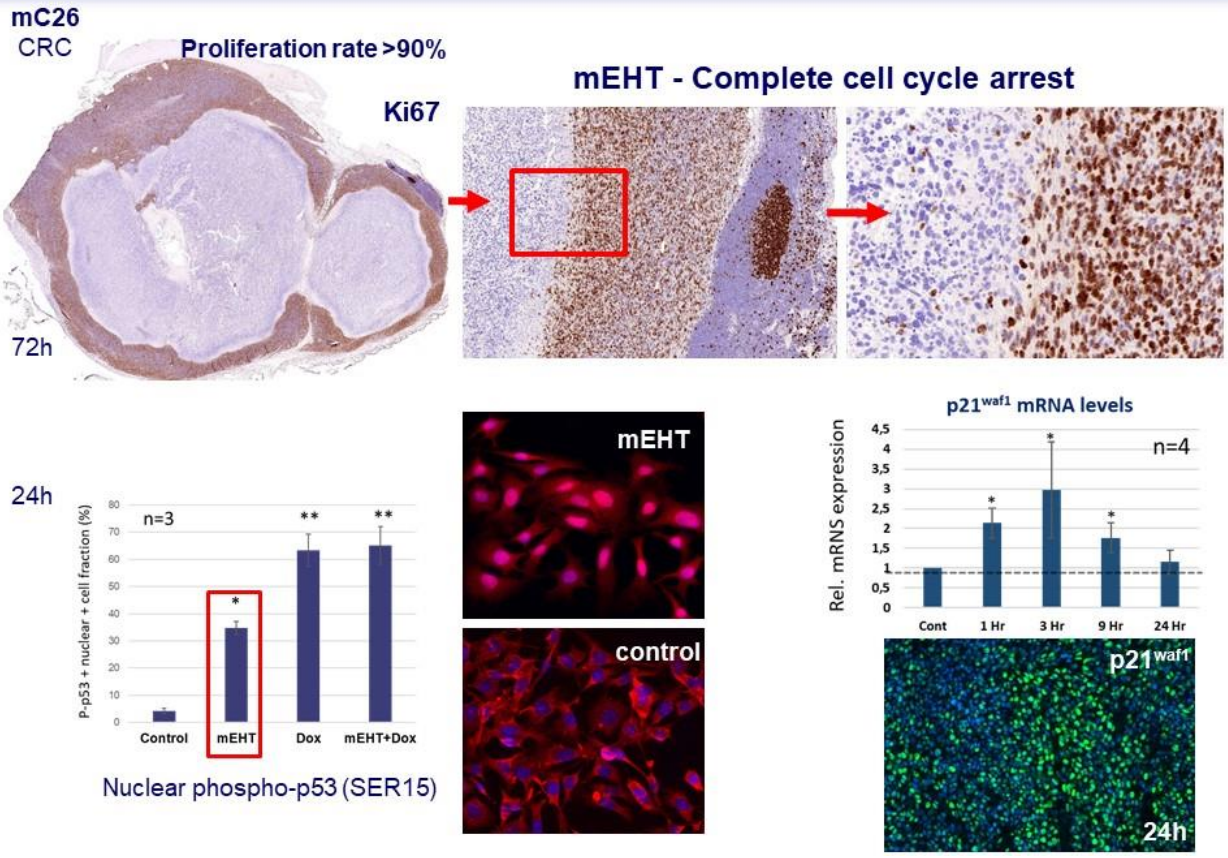
mEHT-iduced DNA double strand brakes



mEHT-iduced DNA damage - apoptosis

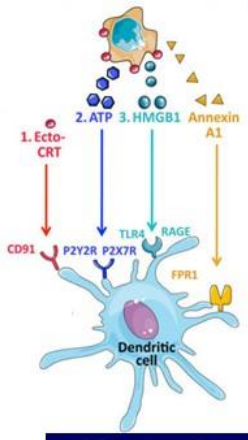


mEHT induced apoptosis and cell cycle arrest



Spatiotemporal „danger" signaling – systhemic effect

Damage associated molecular patterns (DAMP)



Hernandez et al. Oncogene. 2016, 35:5931–5941

- **ATP**
  „find me" signal
- **Calreticulin (CRT)**
  „eat me" signal
- **HMGB1**
  „danger"signal
- **HSP70**
  Granzyme B endocytosis

- DC maturation, activation
- Tumor antigen processing
- T-cell & NK-cell activation

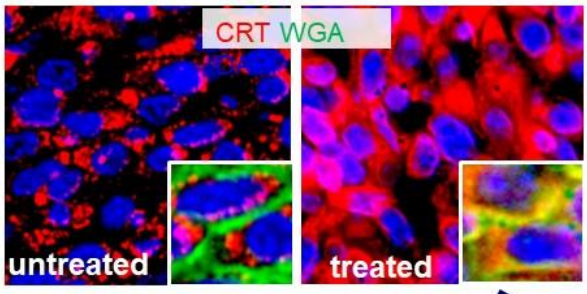
NK cells | CD8+ T cells | CD4+ T cells

- Antitumor immune response
- Immunogenic cell death (ICD)

- **Antracyclins**
  Doxorubicin
- **UV or γ-irradiation**
- **EGFR immunotherapy**
- **Capsaicin**

C26 CRC allograft — Calreticulin membrane translocation

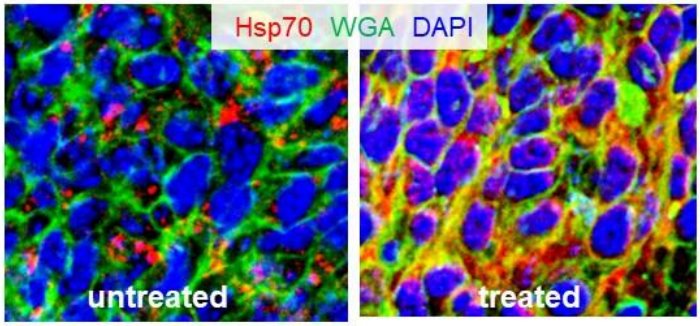
Vancsik et al. (Hungary) J Cancer. 2018, 9:41-53.

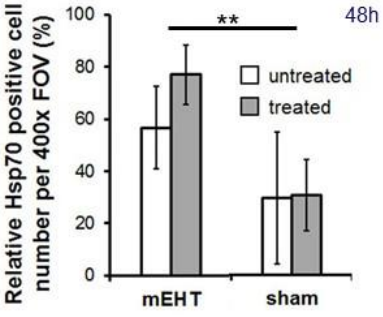


mEHT mC26: CRT *in vitro*

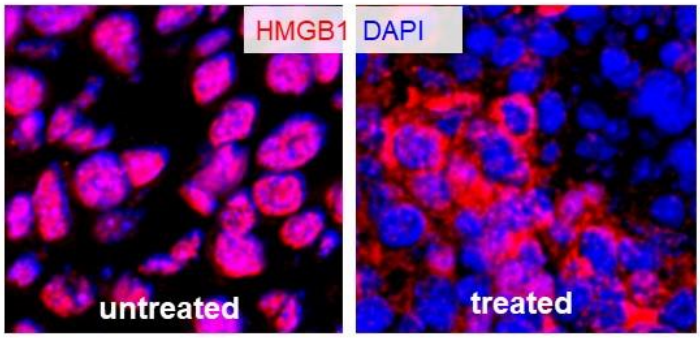
Spatiotemporal DAMP signaling – systhemic effect

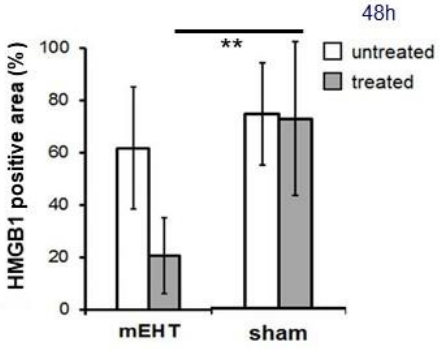
C26 CRC allograft — Hsp70 membrane translocation





HMGB1 release – cytoplasmic & extracellular



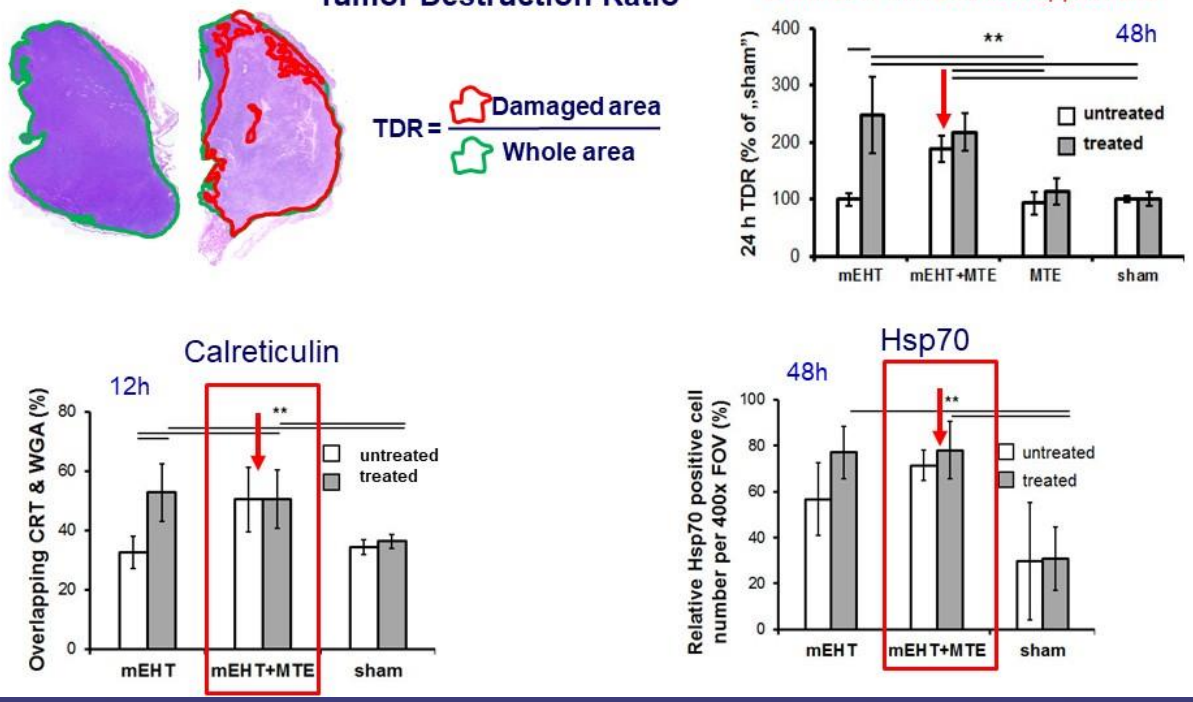


mEHT combined with MTE (T-cell promoter)

Systemic (abscopal) effect

Kang et al. J Anal Methods Chem. 2013;2013:617243.
MTE: Direct antitumor effect + T-cell promotion

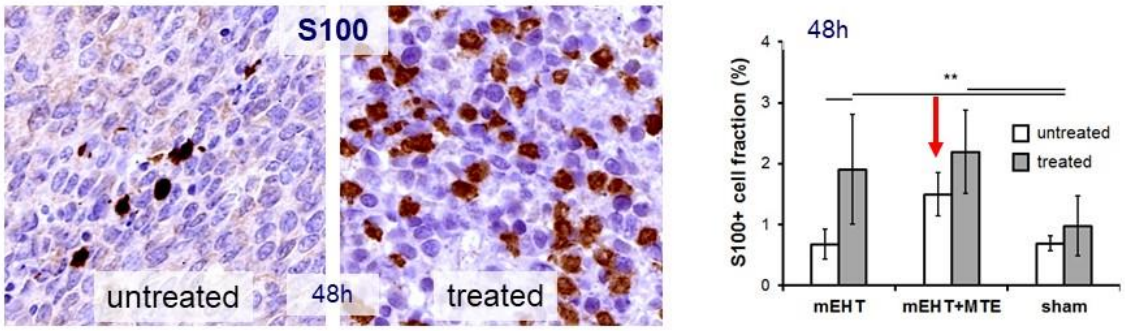
In mEHT tretead & mEHT+MTE treated
& in mEHT+MTE treated opposit site



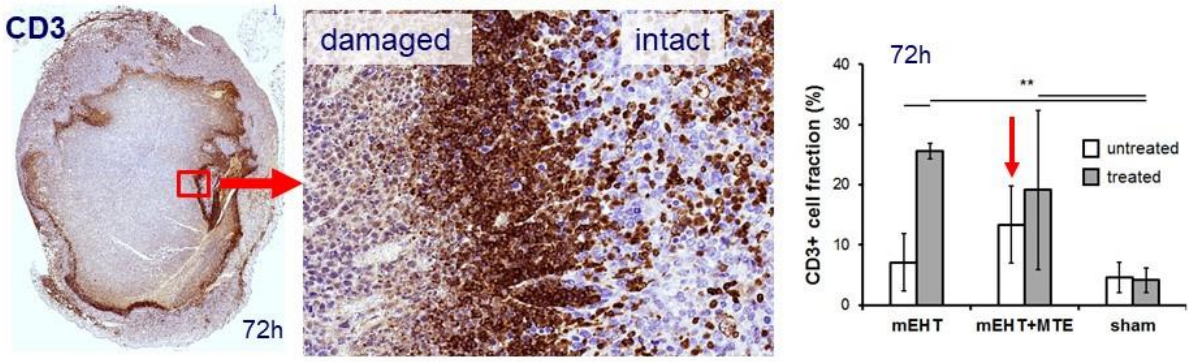
Antitumor immune response - local and systhemic

C26 CRC allograft

Elevated number of antigen presenting DC (APC)



Massive T-cell infiltration

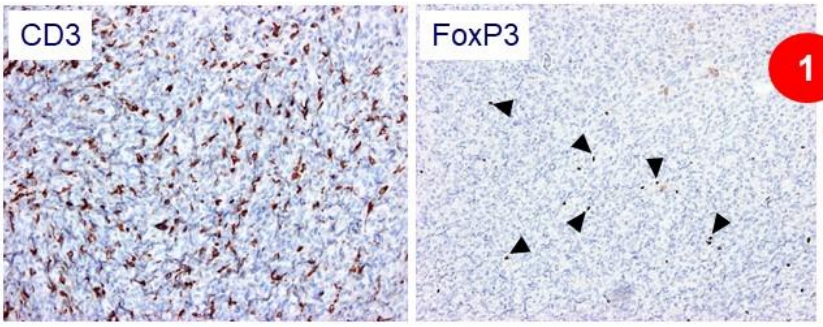


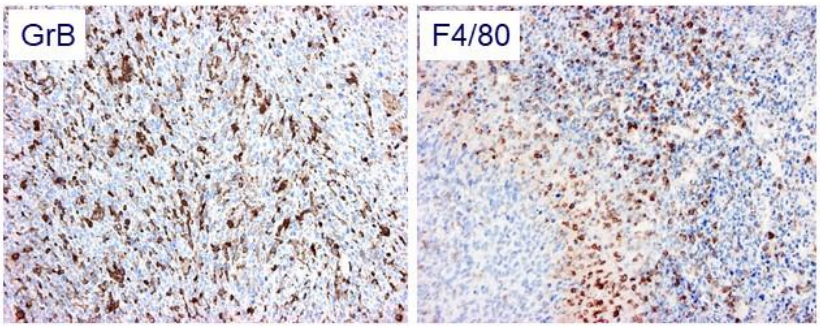
Antitumor immune response – immunogenic cell death

48 h post-mEHT

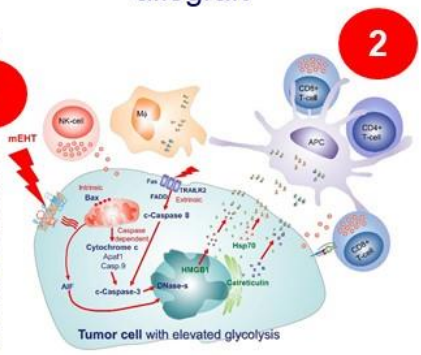
T-cell infiltration, negligible regulatory T-cells

C26 CRC allograft





Cytotoxic T-cells & NK cells + Macrophages



Single mEHT shot

Progressive

- accumulation of immune cells &
- tumor damage

Immunogenic cell death ICD

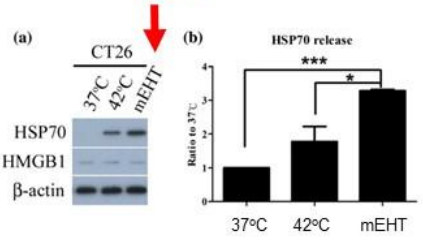
Combination of mEHT + DC therapy

mC26 *in vitro & allografts*

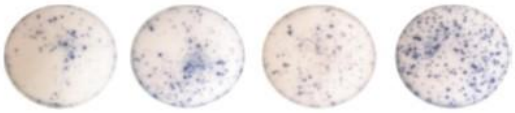
Enhanced immune response

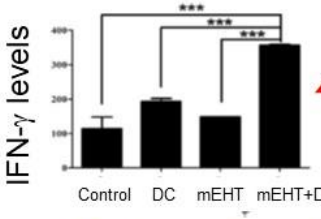
Tsang et al. (Taiwan) BMC Cancer, 2015, 15:708

Elevated Hsp70 release *in vitro*



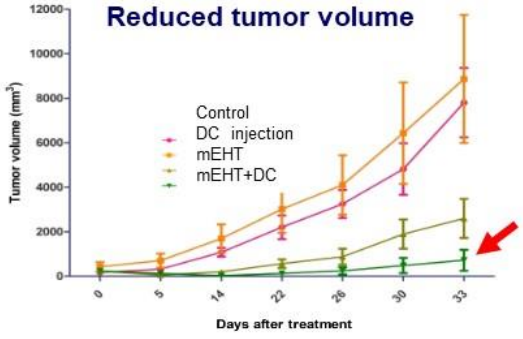
Tumor antigen+Hsp70 activated DC



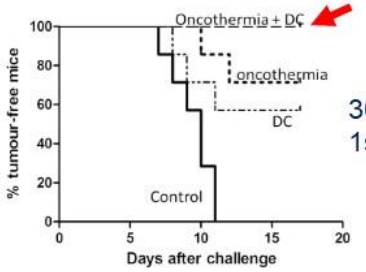


Elevated T-cell activation (& cytotoxicity)

Reduced tumor volume



No tumor after rechallenge



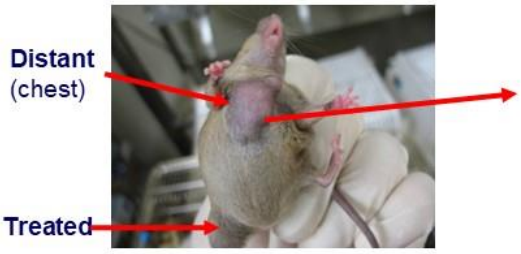
30 days after 1st challenge

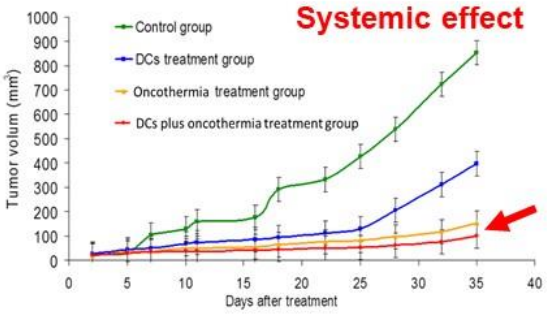
mEHT + DC therapy – systemic „abscopal" effect

Reduced tumor sizes distant from the mEHT treatment site

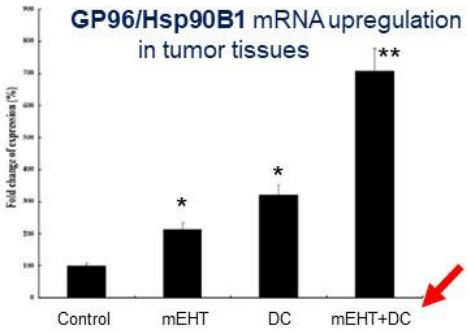
H&N SCCVII allograft

Quin et al. (Japan) Oncology Reports 2014, 32:2373-2379.





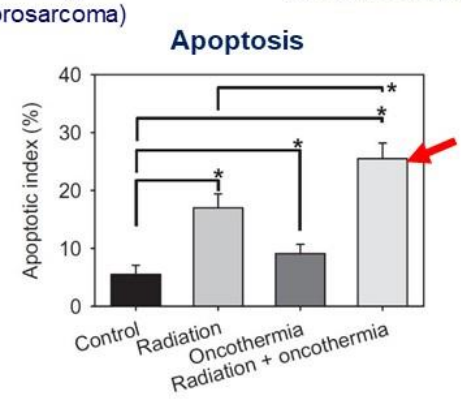
- Elevated CD3+ and CD8+ T-cells & S100+ antigen presenting DCs
- Reduced FoxP3+ regulatory T-cells

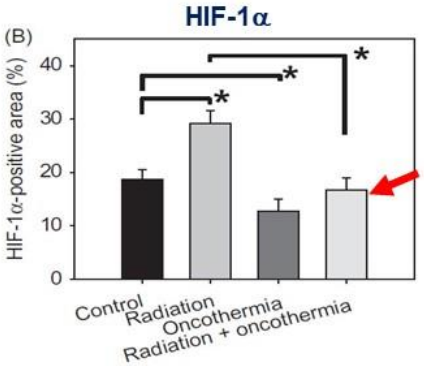


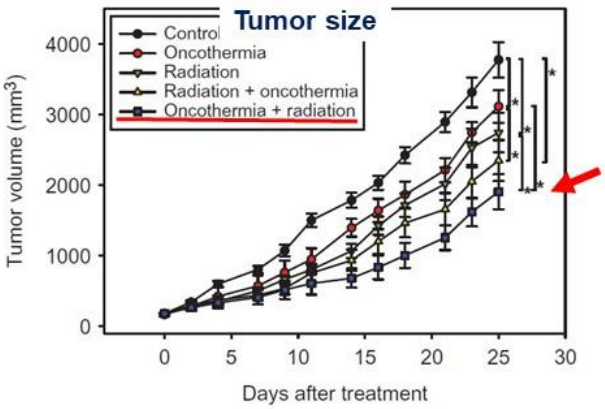
mEHT promoted radiation damage by inhibiting HIF1α

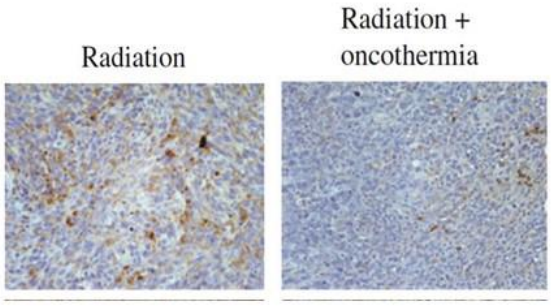
FSall allograft (fibrosarcoma)

Kim et al. (Korea) Int J Hyperthermia. 2018, 34:276-283.







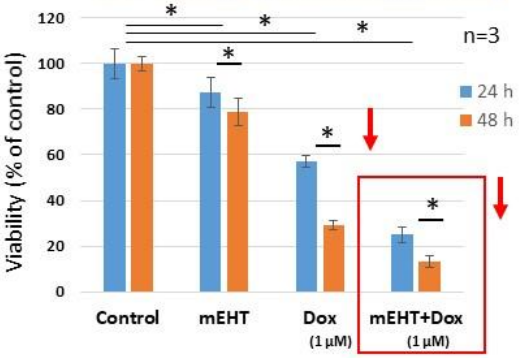


Combination of 2x30' mEHT + Doxorubicin

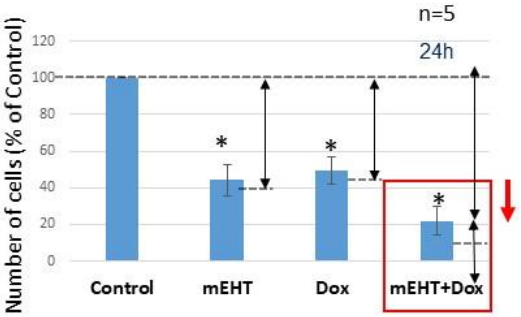
mC26 CRC
in vitro

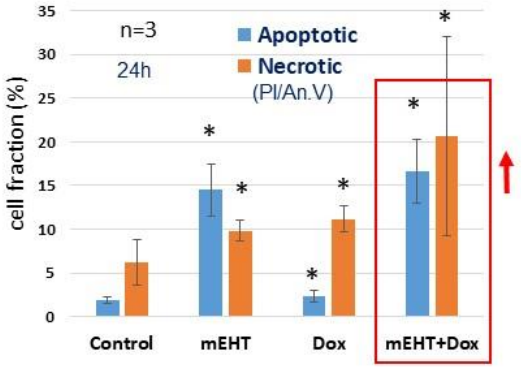
Own group at Semmelweis University (under publication)

Cell viability: Resazurin assay



Cell loss





mEHT - dominantly apoptosis
Doxo - dominantly necrosis

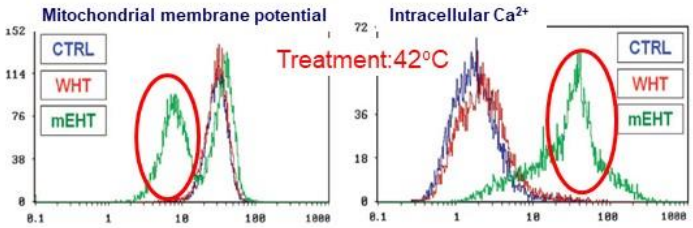
Addiive effect in reducing viability & enhancing cell death by the combination therapy

Comparison of mEHT with conventional HT or cRF therapy

Andocs et al. (Japan) Cell Death Discov. 2016 Jun 13;2:16039.

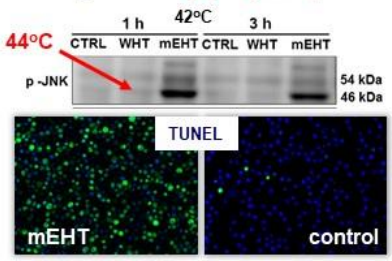
hU937
histiocytic lymphoma

Upregulation of Fas, Casp-8 and Casp-3 & phosphorylation of JNK



mRNA expression array

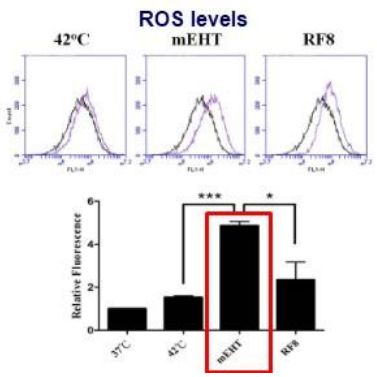
p-JNK → p-p53 (SER6)

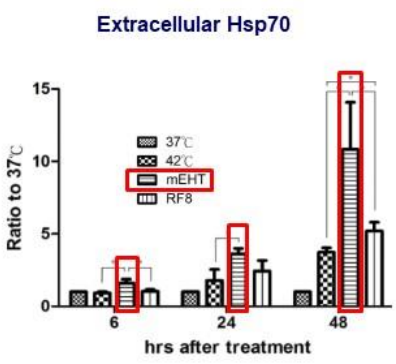


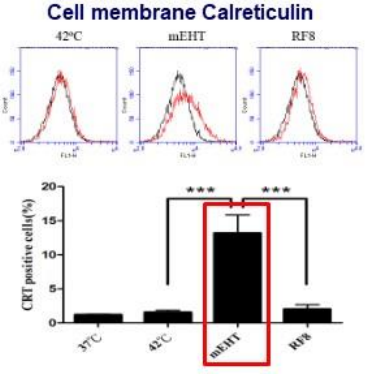
Yang et al. (Taiwan & Japan) Oncotarget. 2016, 7:84082-84092.

hHepG2
HCC

Upregulation of ROS, ex.Hsp70 & CRT; Casp-8 and Casp-3







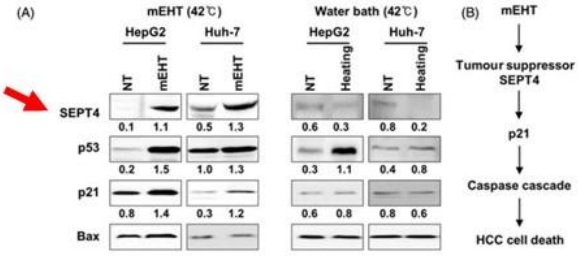
mEHT upregulated Septin-4 promoted p53 functions

HepG2 & Huh7 HCC
in vitro & *in vivo xenograft*

Jeon et al. (Korea) Int J Hyperthermia. 2016, 32:648-56.

Transcriptomic analysis of gene expression by RNA sequencing

Upregulation of Septin-4

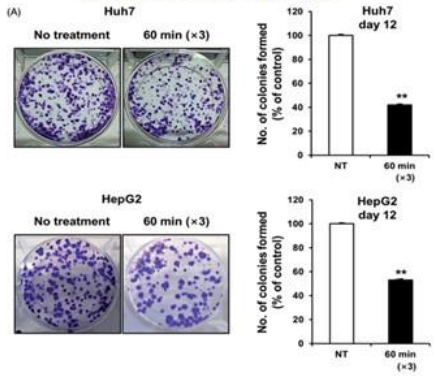


SEPT4 gene, encodes the inhibitor of apoptosis proteins (IAP) antagonist ARTS

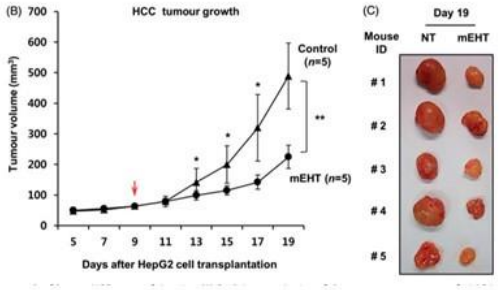
Sept4/ARTS is required for stem cell apoptosis and tumor suppression.

Upregulation of p53

Reduced colony formation



Reduced HepG2 tumor size

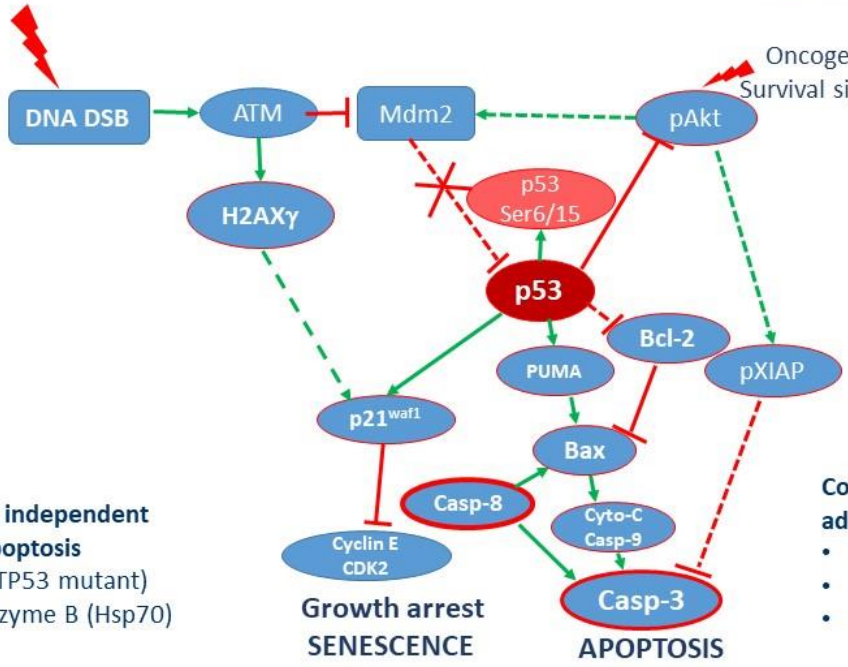


Molecular pathways involved in EHT effects

PRIMARY effect
mEHT induced heath/cell stress

Upregulation, translocation and release of DAMPS

SECONDARY effect
Support of antitumor immune response (ICD)



Caspase independent apoptosis
- AIF (TP53 mutant)
- Granzyme B (Hsp70)

Growth arrest SENESCENCE

APOPTOSIS

Combinations additive effect:
- Dox
- DC
- radiotherapy

Common features of mEHT & Prediction

COMMON

- **The extrinsic apoptosis pathway was involved: cell membrane effect**
- **P53^{wt} activation was frequent: caspase-dependent apoptosis + senescence**

DIFFERENT

- **Extent of tumor damage & the preferred damage signaling pathway(s) are tumor (type) dependent**

& determined by inherent epi-/genetic make up

(the same molecular events in the endogenously damaged areas in controls)

mEHT

PREDICTIVE BIOMAKERS

- **Epi-/genetic predisposition**
- **Oncometabolit** } **levels**
- **Metabolic enzyme** } **levels**

others ???

Acknowledgements

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