

Radiotherapy and modulated electro-hyperthermia effect on Panc1 and Capan1 pancreas adenocarcinoma cell lines

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Radiotherapy and modulated electro-hyperthermia effect on Panc1 and Capan1 pancreas adenocarcinoma cell lines

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Background & Objective

The majority of pancreas malignancies are adenocarcinomas, which show poor outcome. Despite of sophisticated chemotherapy guidelines, those tumor types react very poorly to any treatment regimens, thus new combinations and treatment approaches are intensively searched for. Modulated electro-hyperthermia (mEHT) is a complementary non-invasive cancer treatment modality which uses impedance-coupled radiofrequency to generate selective cell stress and destruction at <42°C in malignant tissue. Here we studied the mechanism of action of mEHT treatment alone and in combination with radiotherapy in Panc-1 and Capan-1, two aggressive pancreas adenocarcinoma cell lines.

Methods

Panc-1 and Capan-1 cells grown on coverslips were treated with mEHT using LabEHY100 (Oncotherm™) for 60 minutes, irradiated with 2 Gy using ¹³⁷Cs source or exposed to combined therapy. To evaluate the effect of treatment on cell death morphological changes were analyzed, apoptosis was measured using Annexin V/ 7-AAD staining, the ALDH⁺ cancer stem cell fraction (CSC) and also the presence of phosphorylated gamma histone H2AX (using both immunocytochemistry and flow cytometry).

Results

Morphological changes (apoptotic bodies, dead cell residues) were observed in both cell lines treated with mEHT. The late apoptotic cell fraction (Annexin V+/7-AAD+) was significantly higher in mEHT alone or 2 Gy + mEHT treated samples than in the irradiated or control groups. The CSC fraction decreased both after mEHT or combined mEHT-radiotherapy treatments, while radiotherapy alone had no remarkable effect on CSC population. The γ-H2AX was upregulated in all treated samples detected by both immunocytochemistry and flow cytometry.

Conclusion

mEHT induced massive apoptosis in both cell lines tested and sensitized cells to radiation. Elevated levels of the double DNA-strand break marker γ-H2AX in mEHT treated samples suggest that the primary mechanism of tumor destruction by mEHT is the induction of DNA lesions, which ultimately lead to apoptosis. Furthermore, mEHT alone or combined with radiation significantly reduced the ALDH⁺ CSC population.

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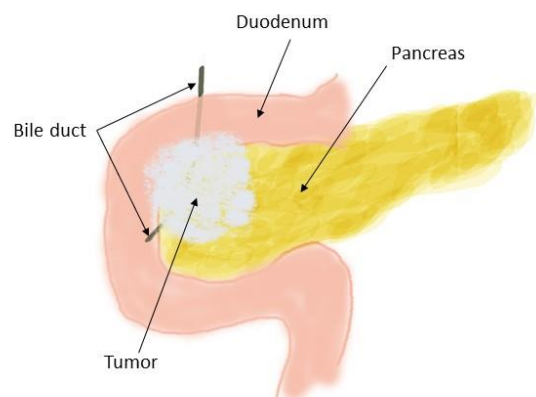
Pancreas malignancies

Statistics:

8,2% survival rate for 5 years
Mortality/incidency index: 98%

Actual treatments:

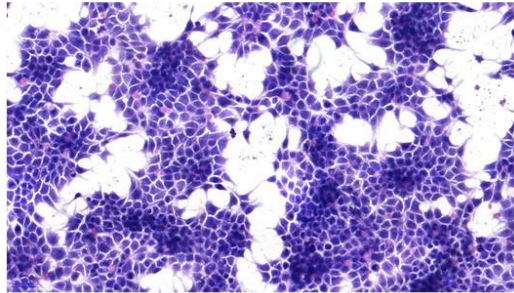
Surgical
Gemcitabine, Erlotinib, FOLFIRINOX



Pancreas adenocarcinoma cell lines

Panc1:

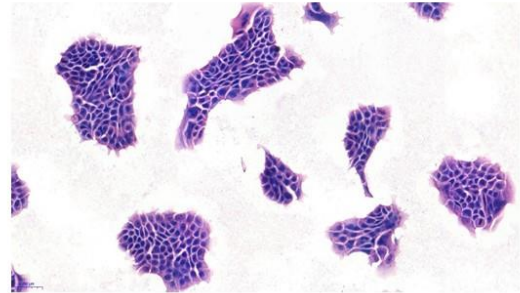
1975 isolated from a ductal adenocarcinoma
Good model for radio-chemoresistivity
Numerous metastases *in vivo*
High tumor stem cell rate



Haematoxylin eosin staining, Ob:20x

Capan1:

Isolated from a liver metastasis of pancreatic adenocarcinoma
Good model for radio-chemoresistivity

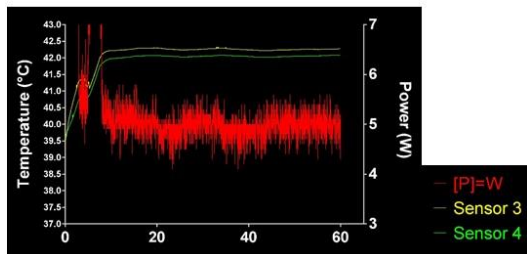


Haematoxylin eosin staining, Ob:20x

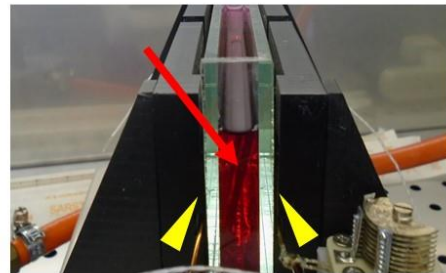
mEHT treatment *in vitro*

Modulated electro-hipertermia (mEHT):

- Complementer therapy to radio- or chemotherapy
- Non invasive
- 13.56 MHz radiofrequency -> electric field => 42°C heat
- Selective: elevated glycolysis, ion concentration and conductivity

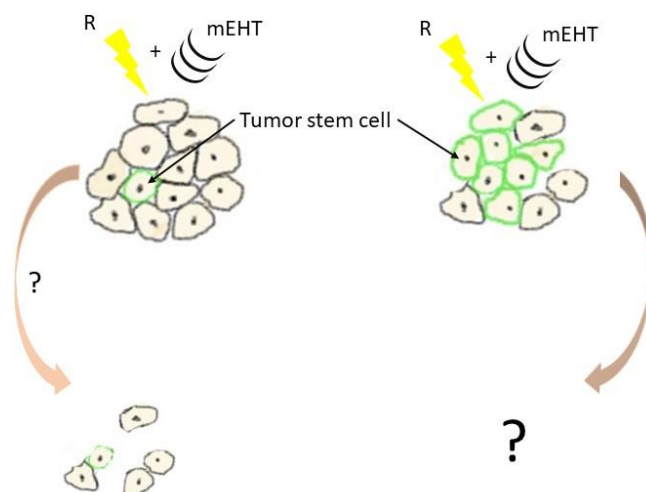


Heat control with power adjustment



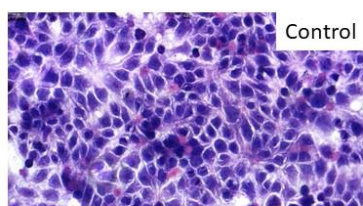
Aim of the work

- Is mEHT treatment effective on Panc1 and Capan1 cell lines?
- Can mEHT treatment support radiotherapy on tumor cell destruction?
- Combination therapy is effective on tumor stem cells too?

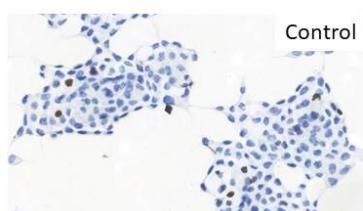
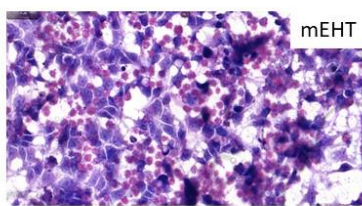


60 min mEHT treatment – Panc1

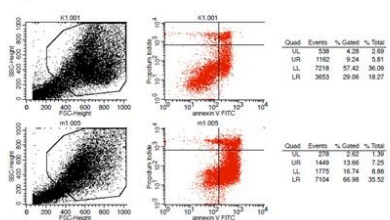
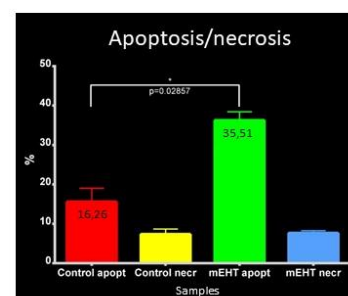
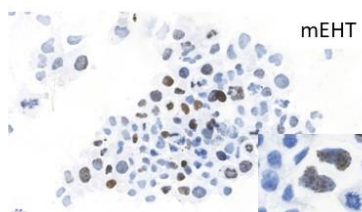
Cell destruction after 24 hours



HE - 24 H



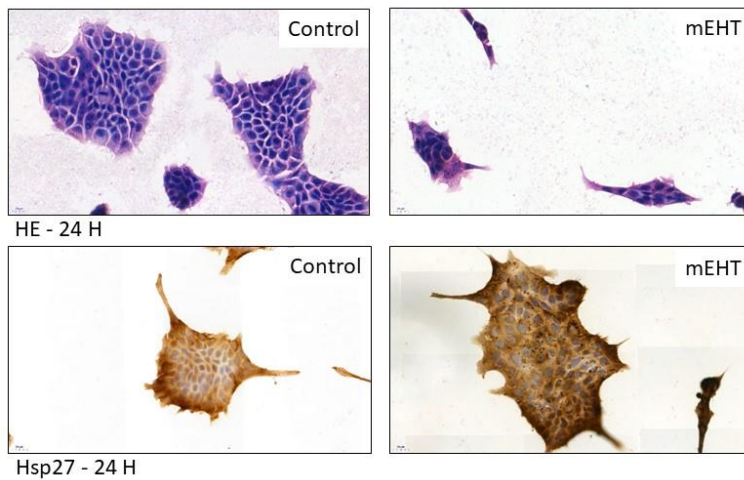
γH2Ax - 24 H



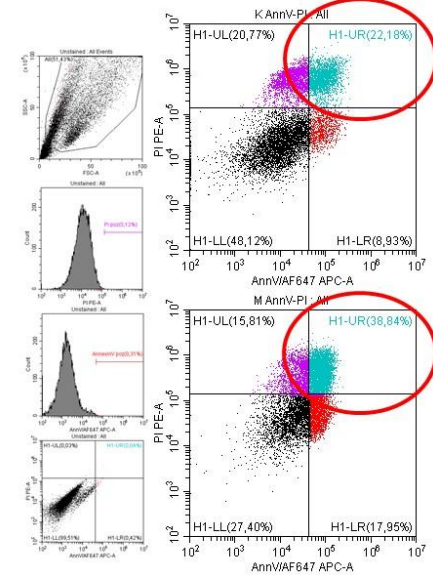
Annexin V and Propidium iodide staining

60 min mEHT treatment – Capan1

Cell destruction and cell stress after 24 hours



Annexin V and Propidium iodide staining

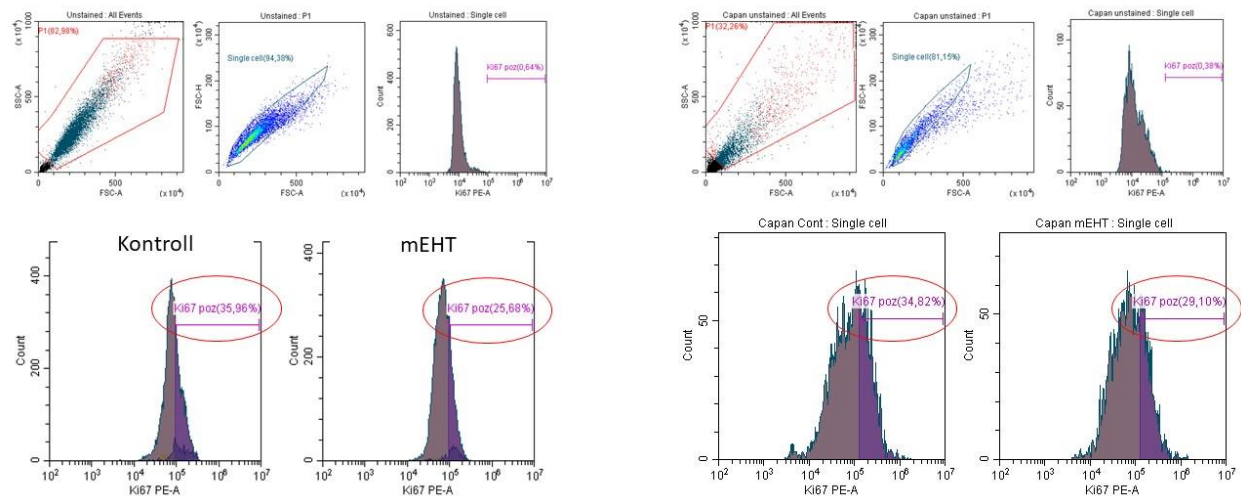


60 min mEHT treatment – Ki67

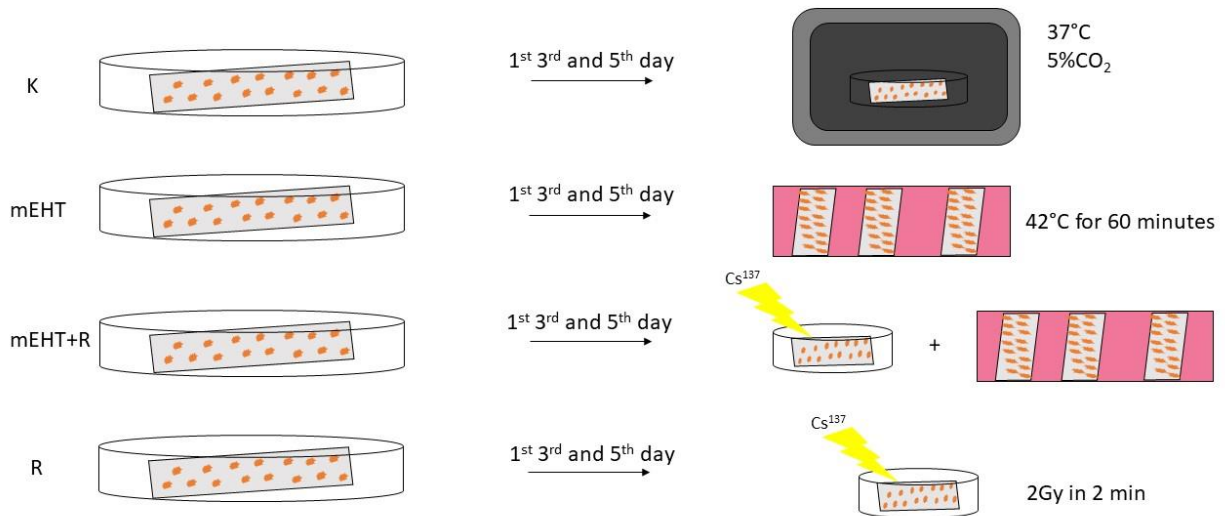
Panc1

Capan1

Ki67: proliferation marker – present in active cell cycle

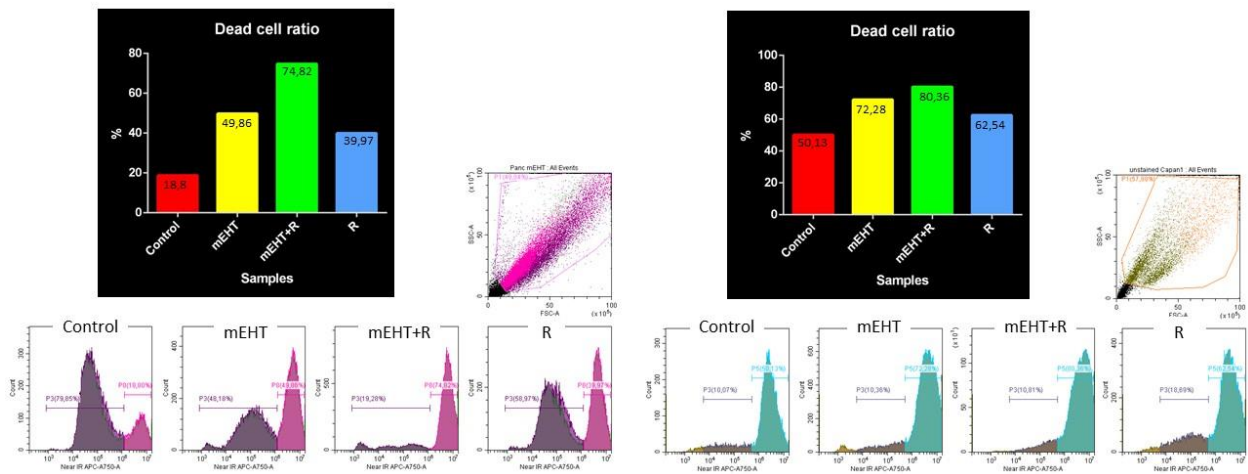


Combination treatment: 60 min mEHT + 2Gy radiotherapy 3×



Combined treatment: Live/dead cell rate Panc1 Capan1

LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit - two population: weak signal = living cells, strong signal = dead cells

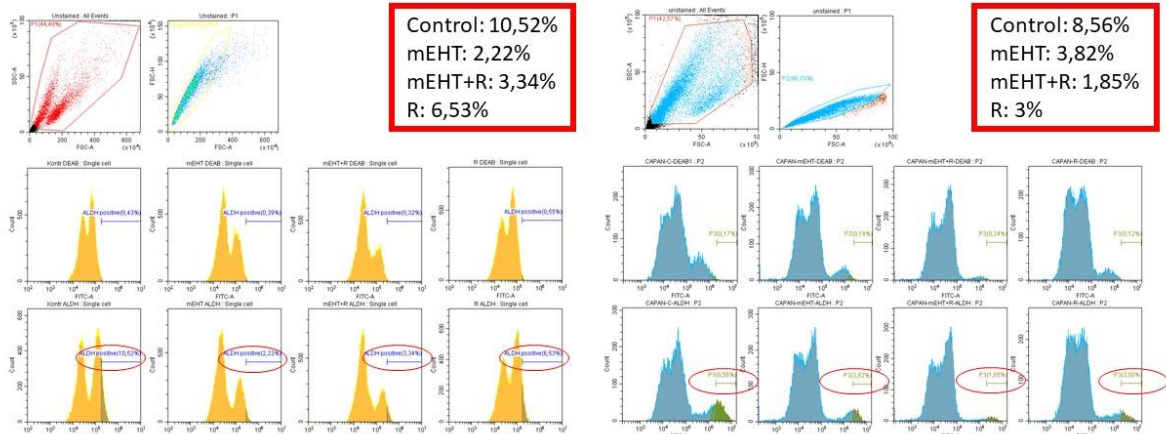


Combined treatment: Stem cell ratio

Panc1

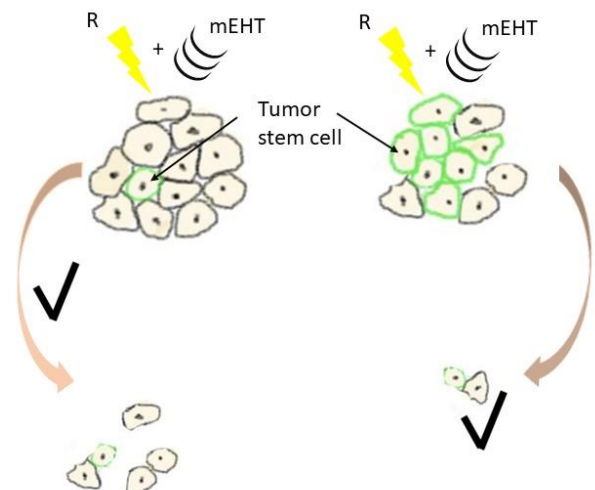
Capan1


ALDH – aldehyde dehydrogenase – highly expressed by tumor stem cells
 ALDEFLUOR Kit – using to detect the ALDH expressing cell amount by flow cytometry



Conclusions

- Both cell lines (Panc1, Capan1) are suitable for mEHT treatment study
- 1X60 minutes mEHT can lead to a massive apoptosis and cell stress
- Combined with radiotherapy, mEHT potentiate the effectivity of the treatment
- Tumor stem cells are sensitive for mEHT or for combined treatment despite of their resistance for the radiotherapy alone





Thank you for your attention

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- Vancsik Tamás

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