Apoptotic response and DNA damage of the radioresistant Panc1 pancreas adenocarcinoma to combined modulated electro-hyperthermia and radiotherapy

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Abstract

The pancreas ductal adenocarcinomas (PDAC) have a poor prognosis, due to the high resistance to standard therapies. Modulated electro-hyperthermia (mEHT) generated by 13.56 MHz capacitive radiofrequency can induce direct tumor damage and promote chemo- and radiotherapy. In this study, we tested the effect of mEHT either alone or in combination with radiotherapy using an in vitro model of Panc1, radioresistant PDAC cell line. A single mEHT shot of 60 min induced ~50% loss of viable cells and morphological signs of apoptosis including chromatin condensation, nuclear shrinkage and apoptotic bodies. The mEHT treatment related effects were more expressive when the cells were pretreated with 2Gy radiotherapy. Treatment related apoptosis was confirmed by a significantly elevated number of annexin V single-positive and cleaved/activated caspase-3 positive tumor cells, as well as sub-G1-phase tumor cell fractions. mEHT and mEHT+radioterapy caused the moderate accumulation of H2AX positive nuclear foci, indicating DNA double-strand breaks and upregulation of the cyclin dependent kinase inhibitor p21waf1 besides the downregulation of Akt signaling. A clonogenic assay revealed a tumor progenitor/stem cell loss too. In conclusion, mEHT treatment can contribute to tumor growth inhibition and apoptosis induction and resolves radioresistance of Panc1 PDAC cells.

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The radioresistant Panc1 pancreas adenocarcinoma answers with apoptosis and DNA damage to modulated electro-hyperthermia treatment

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Hungary had the highest rate of **pancreatic cancer** in 2018 age-standardized rate per 100,000. Just 10% of diagnosed pancreas ductal adenocarcinomas are suitable for surgical resection. For unresecable tumors chemo- and/or the radiotherapy are used as treatment possibility, unfortunately with poor outcome due to **therapy resistance**.

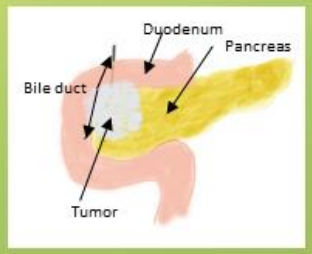
Pancreatic cancer rates: both sexes

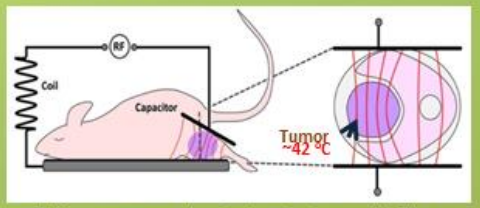
Hungary had the highest rate of pancreatic cancer in 2018, followed by Uruguay.

Rank	Country	Age-standardised rate per 100,000
1	Hungary	10.8
2	Uruguay	10.7
3	Moldova	10.5
3	Latvia	10.3
5	Japan	9.7

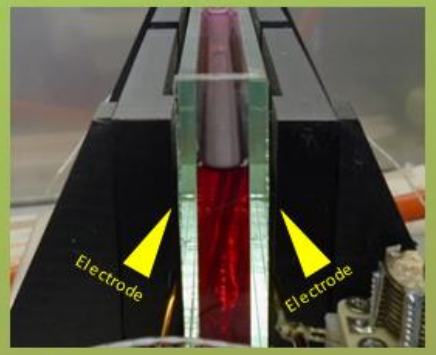
Source: https://www.wcrf.org/dietandcancer/cancer-trends/pancreatic-cancer-statistics

Modulated electro-hyperthermia delivers loco-regional deep hyperthermia by using 13.56 MHz radiofrequency. The generated electric field can be accumulated in malignant tumors to induce selective temperature increase (around 42°C), as a result of elevated glycolysis, lactate concentration and electric conductivity there compared to the adjacent tissues.

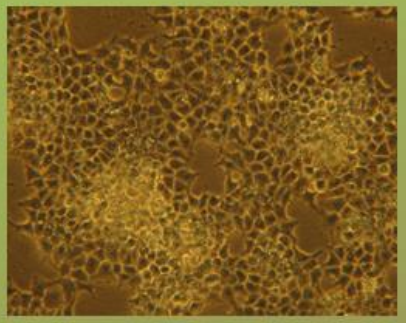




mEHT treatment mechanism in an in vivo model. The elevated ion concentration on the tumor, accumulates the elecrtic field and generates local hyperthermia



Lab-EHY 100 device electrode system for *in vitro* mEHT treatment



Panc1 pancreas adenocarcinoma cell cluture on light microscope (Ob.: 40×)

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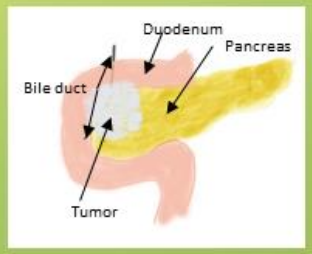

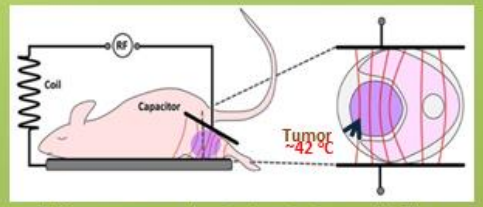

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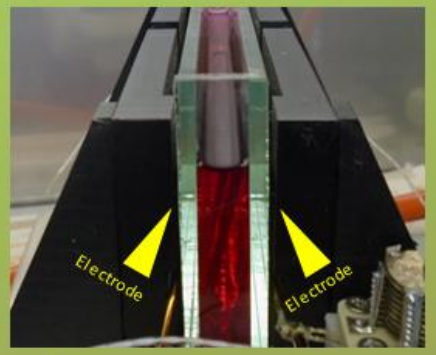

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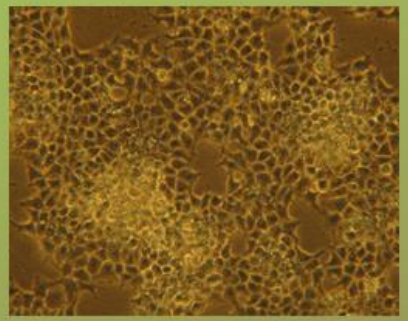

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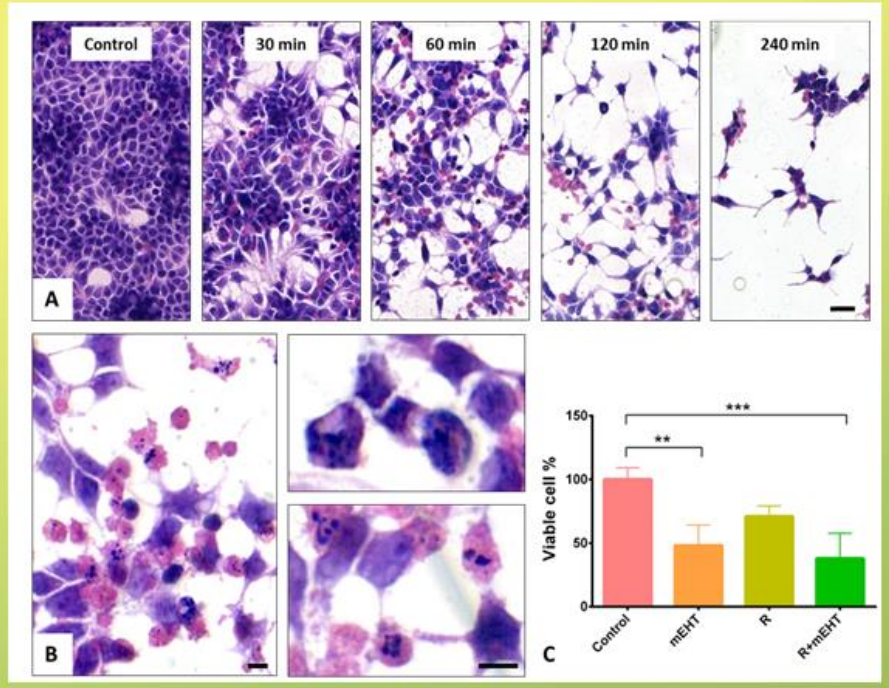
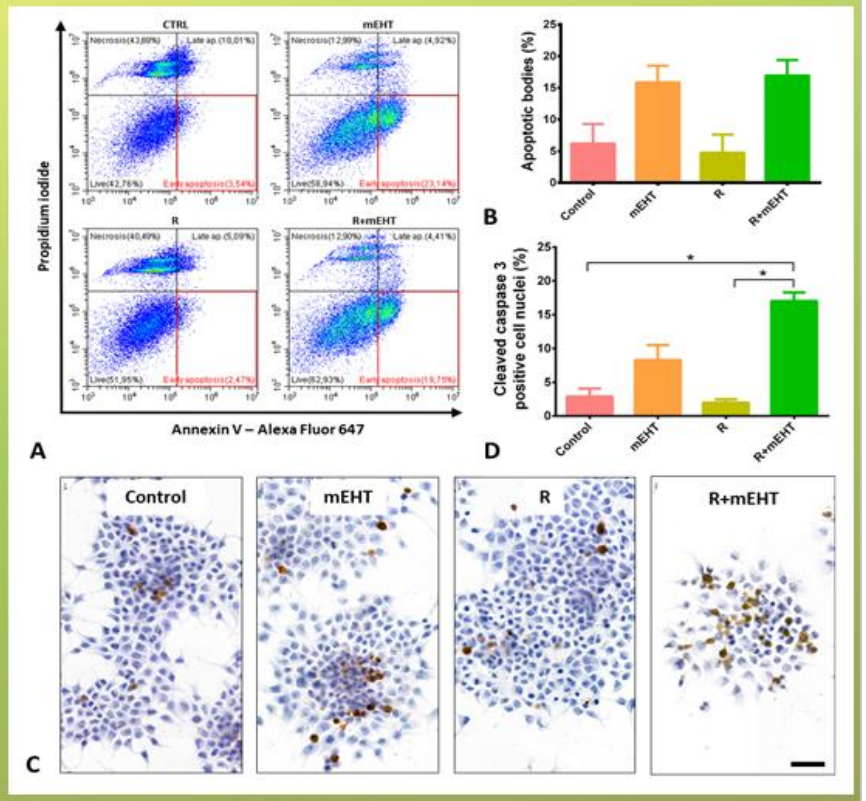


Hematoxylin and eosin staining of Panc1 cell cultures demonstrating **cell loss** and the major changes in cell morphology 24 h after different mEHT treatment durations (A). Scale bar: 40 µm. Higher magnifications reveal nuclear shrinkage, chromatin condensation (upper right), and apoptotic bodies (lower right) after 60 min treatment (B). Scale bars: 10 µm.



Cleaved/activated caspase-3 immunoreactions (C) (scale bar = 50µm) and graphical representation of its results (D).

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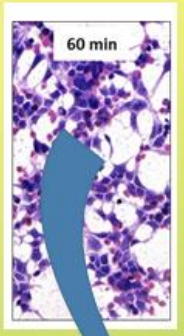
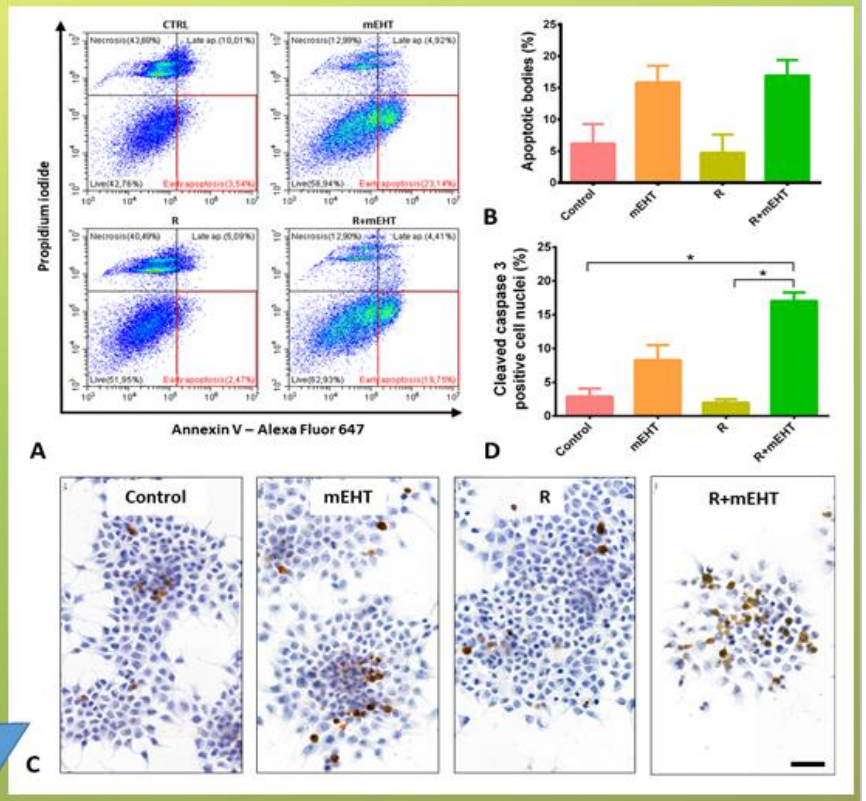


Apoptosis dominated programmed cell death induced by **60 min mEHT** in Panc1 cultures. Flow cytometry results of **Annexin V and propidium iodide** double stained tumor cells 24 hours after treatments (A). Graphical representation of early apoptotic bodies in proportions of the whole population.



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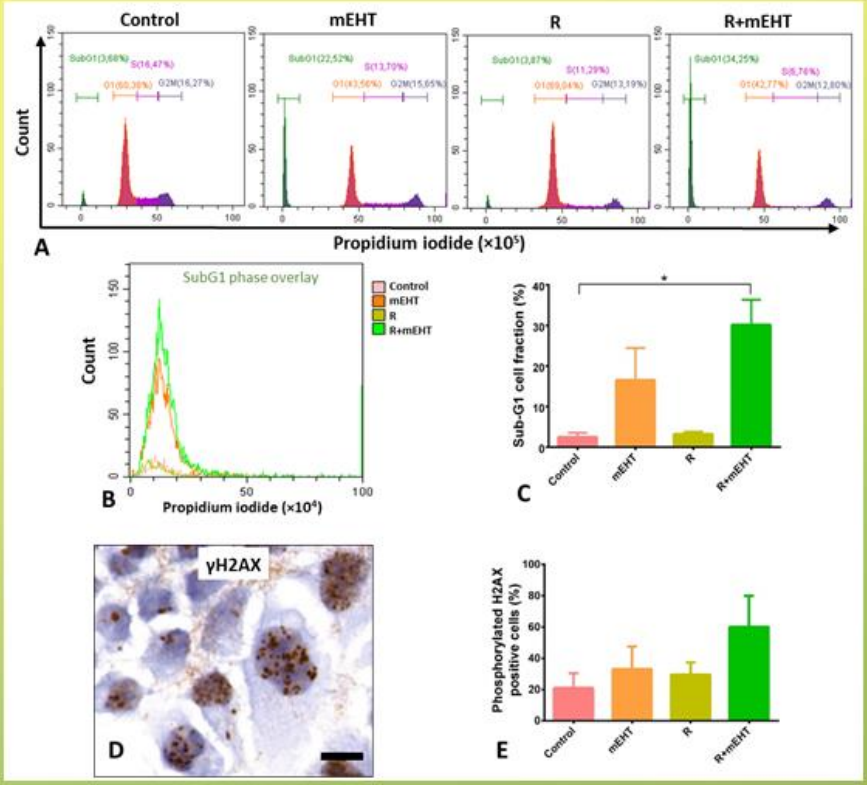

Treatment related elevated **subG1-fractions** and moderate increase in **nuclear phosphorylated H2AX protein** levels in Panc1 cultures 24 h post-treatment.

The paper is published and it's accesible from: https://www.mdpi.com/1422-0067/21/14/5100/htm

Open Access Article

Modulated Electro-Hyperthermia Resolves Radioresistance of Panc1 Pancreas Adenocarcinoma and Promotes DNA Damage and Apoptosis In Vitro

by Gertrud Forika [1], Andrea Balogh [2], Tamas Vancsik [2], Attila Zalatnai [1], Gabor Petovari [1], Zoltan Benyo [2] and Tibor Krenacs [1,*]

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Abstract

The poor outcome of pancreas ductal adenocarcinomas (PDAC) is frequently linked to therapy resistance. Modulated electro-hyperthermia (mEHT) generated by 13.56 MHz capacitive radiofrequency can induce direct tumor damage and promote chemo- and radiotherapy. Here, we tested the effect of mEHT either alone or in combination with radiotherapy using an in vivo model of Panc1, a KRAS and TP53 mutant, radioresistant PDAC cell line. A single mEHT shot of 60 min induced ~50% loss of viable cells and morphological signs of apoptosis including chromatin condensation, nuclear shrinkage and apoptotic bodies. Most mEHT treatment related effects exceeded those of radiotherapy, and these were further amplified after combining the two modalities. Treatment related apoptosis was confirmed by a significantly elevated number of annexin V single-positive and cleaved/activated caspase-3 positive tumor cells, as well as sub-G1-phase tumor cell fractions. mEHT and mEHT+radioterapy caused the moderate accumulation of γH2AX positive nuclear foci, indicating DNA double-strand breaks and upregulation of the cyclin dependent kinase inhibitor $p21^{waf1}$ besides the downregulation of Akt signaling. A clonogenic assay revealed that both mono- and combined treatments affected the tumor progenitor/stem cell populations too. In conclusion, mEHT treatment can contribute to tumor growth inhibition and apoptosis induction and resolve radioresistance of Panc1 PDAC cells. View Full-Text