Elevated apoptosis and tumor stem cell destruction in a radioresistant pancreatic adenocarcinoma cell line when radiotherapy is combined with modulated electrohyperthermia

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Presented at the 33rd ESHO, Warsaw

Cite this article as:
Forika G. et al. (2019) Elevated apoptosis and tumor stem cell destruction in a radioresistant pancreatic adenocarcinoma cell line when radiotherapy is combined with modulated electrohyperthermia. Oncothermia Journal 26:90-98
Objective: Malignant exocrine tumors of the pancreas are among the worst to respond to oncotherapy. Despite sophisticated guidelines and new targeted therapies, the 5-year survival rate of patients with pancreatic adenocarcinomas is under 10%. The most critical factor responsible for this is the high resistance of the tumor cells to the available chemo- or radiotherapies.

Modulated electro-hyperthermia (mEHT) is a complementary non-invasive cancer treatment using impedance-coupled radiofrequency to generate selective heat of <42°C causing cell stress and destruction in malignant tissue. In this study, we tested the combination of radiotherapy with mEHT in a radioresistant pancreatic adenocarcinoma cell line Panc1.

Methods: Panc1 adherent cells grown on coverslips were used to create 3 parallels in 4 groups: control (C); mEHT treated for 60 min (mEHT); irradiated with 2 Gy using $^{137}$Cs (R), and combination treatment: irradiation followed by the same dose of mEHT (mEHT+R). 24 hours after treatments we observed the cells morphology, the proportion of apoptotic and necrotic (AnnexinV/propidium iodide positive) cells, the ALDH+ tumor stem cell (CSC), the colony forming capacity of cells, the H2Axy positivity and the calreticulin presence in the cells. For quantitative and semiquantitative analysis we used: hematoxylin-eosin staining, flow cytometry and immunocytochemistry.

Results: Visible morphological changes were observed after 24 hours in the treated groups: an elevated number of apoptotic bodies and cell number loss. Compared to the control group, the apoptotic ratio was the highest in the mEHT+R group and significant elevation was measured also in the mEHT group. ALDH+ tumor stem cells decreased significantly after mEHT and mEHT+R treated groups compared to the control. As it was expected the irradiated group showed the same amount of CSC cells as the control group (due to well-known radioresistance of the cell line). The CSCs colony forming capacity was also significantly lower in the mEHT and mEHT+R group compared to the control group. Furthermore, H2Axy and calreticulin positive cell fractions, indicating DNA double strand-brakes and ER-stress, respectively, were also significantly increased in the mEHT and the mEHT+R treated groups.

Conclusion: mEHT treatment alone can lead to massive apoptosis in Panc1 cells by inducing cell stress and DNA double-strand break. Irradiation alone caused some necrosis but without major effect on CSCs. The combined treatment significantly improved the efficacy of radiotherapy resulting in major apoptosis and reduction of CSCs despite of the inherent radioresistance of Pan1.

This study was funded by a grant of the National Research and Innovation Office (NKFIH- NVKP_16-1-2016-0042) in Hungary.
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Pancreas adenocarcinomas - statistics

- Hungary had the highest rate of pancreatic cancer in 2018 age-standardised rate per 100,000 (https://www.wcrf.org/dietandcancer/cancer-trends/pancreatic-cancer-
  incidence)
- Pancreatic cancer is the 5th common cause of death in Europe (https://ec.europa.eu/health/news)
- The 5 year survival rate is under 10% (https://seer.cancer.gov/statfacts/html/pancreas.html)
Pancreas adenocarcinomas - treatment

• The cancer stages at diagnosis are not promising \(^{(1)}\)

• Just 10% of patients diagnosed with pancreatic cancer in England during 2013-2014 had surgery to remove their primary tumour, as part of their primary cancer treatment \(^{(4)}\)

• Commonly used chemotherapy drugs:
  • Paclitaxel
  • 5FU / 5 Fluorouracil
  • Gemcitabine Hydrochloride
  • Irinotecan

• Combination: FOLFIRINOX
• Targeted therapy: Erlotinib (Tyrosine Kinase Inhibitor)
• Radiotherapy
• Radiochemotherapy

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Modulated electro-hyperthermia

• **Regional** – deep tissue hyperthermia
• **Complementer** therapy to radio- or chemotherapy
• **Non invasive**

• 13.56 MHz radiofrequency -> electric field -> 42°C heat
• Selective: elevated glycolysis, ion concentration and conductibility (Warburg effect)
Material and methods

Cell line: Panc1
- Isolated from a ductal adenocarcinoma,
- 56-year-old male in 1975
- Epithelial morphology
- High tumorigenicity

Methods: treatment protocol

- Control
- mEHT
- R+mEHT
- R

- Radiotherapy
- mEHT treatment

- Heat control with power adjustment

- 37°C 5%CO₂
- 42°C for 60 minutes
- Cs¹³¹
- 2Gy in 2 min
Results: apoptosis/necrosis

Cell numbers counted with trypan blue dye after treatments

Apoptotic/necrotic rate analysed with flow cytometry. Used staining: Annexin V and propidium iodide

The bar graph on the left shows the cell number distribution across control, mEHT, R, and R+mEHT treatments. The bar graph on the right displays the apoptotic and necrotic percentage distribution in the same treatment groups.
Results: apoptosis

Cleaved caspase 3 positivity

Presumably the apoptosis is caspase 3 dependent. The immunocytochemistry reaction showed many positive nuclei in the mEHT treated groups.

Results: DNA double strand breaks

Phosphorilated H2AX positivity

The phosphorilated H2AX is a marker of DNA double strand breaks.
Results: tumor progenitor cells

ALDH+

ALDH – aldehyde dehydrogenase is highly expressed by tumor progenitor cells

Percentage of ALDH positive cells →

Colony forming capacity
Tumor progenitor cells has the capacity to form colonies

Conclusion

• 60 minutes mEHT can lead to a massive apoptosis
• Combined with radiotherapy, mEHT potentiate the effectivity of the treatment
• Tumor stem cells are sensitive for mEHT or for combined treatment despite of their inherent radioresistance
• The mEHT treatment leads to caspase dependent apoptosis
• Presumably the mEHT has negative effect on DNA repair
Thank you

- Mátrainé Balogh Éva
- Vancsik Tamás
- Balogh Andrea

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