Modulated electro-hyperthermia treatment of pancreas ductal adenocarcioma in vitro

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Modulated electro-hyperthermia treatment of pancreas ductal adenocarcinoma in vitro

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Introduction:

Pancreas adenocarcinomas still show very poor survival rates despite of up-to-date chemotherapy. Pancreatic cancer is the 7th most lethal cancer type worldwide. According to SEER the survival rate for 5 years is only 8.5% which reflects the insufficient treatment options in pancreatic cancers.

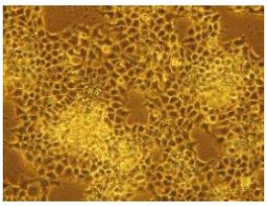


Figure 1. *Confluent Panc1 pancreatic adenocarcinoma cell line growth in cell culture flask. x40*

Modulated electro-hyperthermia (mEHT) is a complementary tumor therapy utilizing capacitive, impedance-coupled radiofrequency for inducing cell stress at ~42°C. It can selectively target malignancies due to their elevated glycolysis, ion concentration and conductivity compared to normal tissues. In this work we have analyzed the effect of mEHT therapy in Panc1 pancreas ductal adenocarcinoma.

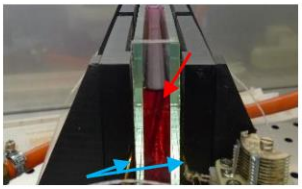


Figure 2. *The mEHT applicator set up with 4 coverslip cultures side-by-side in vertical position inside the tank (red arrow) embraced by electrodes from both sides (blue arrows).*

Material and methods:

Panc1 cells growth to confluency on coverslips in Dulbecco Modified Eagle Medium (DMEM) were treated using mEHT (LAB-EHY 100 device). For analyzing morphologycal changes, the cells were fixed in metahanol-acetone on coverslips and stained with hematoxyline & eosine (H&E) staining. Apoptotic/necrotic ratio was determined using flow cytometry of the whole cell population after labelling with annexin V and propidium iodide (PI). mEHT induced cell stress markers including the H2Axγ (for DNA double straind break), the chaperone calreticuline and the apoptosis mediator and exosome indicator Alix proteins were tested *in situ* using immunocytochemistry.

Results:

Single mEHT treatment for 60 minutes resulted massive tumor-cell damage after 24 h with the accumulation of largre numbers of apoptotic bodies.

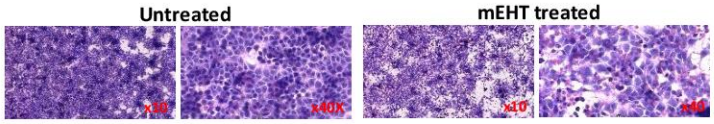
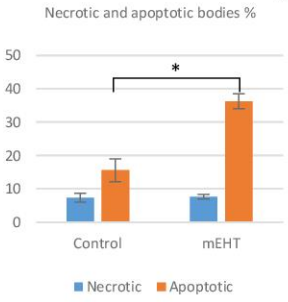


Figure 3. *H&E staining of cell cultures 24 h after mEHT treatment. The control group is dominated by live cells, while there are large numbers of damaged cells with apoptotic bodies and cell debrie in the treated group.*

Flow cytometry using AnnexinV/PI labeling confirmed significant elevated apoptotic ratio in the treated group compared to the controls (***Figure 4***)



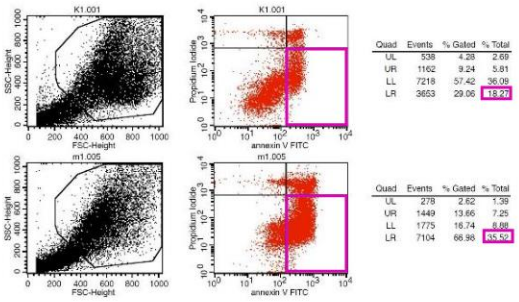


Figure 4. *Flow cytometry of annexin V/PI labelled cells (B) and graphic illustration of the differences (A) with signifficant elevated apoptotic body fraction in the treated group.*

After mEHT treatment immunocytochemistry revealed elevated numbers of histone 2 Axγ positive tumor cells indicating increased DNA double-strand breaks. Also, the treatment caused increased expression of Alix protein related to apoptosis and exosome formation and that of the cell stress indicator calreticulin protein (***Figure 5***).

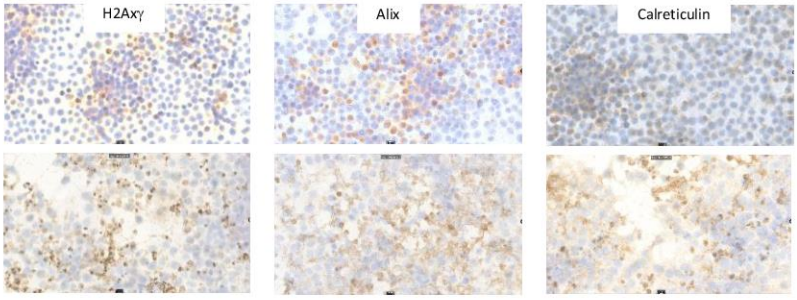


Figure 5. *The results of immunocytochemical stress marker detection in Panc1 cultures 24 hours after mEHT treatment.*

Conclusion: Our results show that mEHT treatment can induce an efficient tumor cell stress and massive apoptosis involving significant DNA double strand brakes in pancreas adenocarcinoma cells. Further studies are in progress to clarify damage mechanisms and apoptotic pathways for translating knowledge into *in vivo* animal experiments and human mEHT therapy.

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