The effects of Low Intensity Ultrasounds (LIUS) on tumor and fibroblast cells in vitro as a novel strategy in the treatment of pancreatic cancer.

Jesús Frutos Díaz-Alejo[1], Iciar González Gómez[2] Alfredo Carrato[1] and Julie Earl[1].

[1]*Molecular Epidemiology and Predictive tumor markers group, Ramón y Cajal Health Research Institute (IRYCIS), Carretera Colmenar Km 9,100. 28034. Madrid, Spain.*

[2] *Institute of Physical and Information Technologies (ITEFI), CSIC, C/ Serrano, 144, 28020 Madrid, Spain.*

Introduction: In 2030, adenocarcinoma of the exocrine pancreas (PDAC) will be the second leading cause of cancer death, after lung cancer and the overall survival at 5 years is around 7.2%. There have been few clinical advances in PDAC treatment over the last 20 years and chemotherapy is the main treatment option as these tumors are resistant to many therapies due to the presence of a desmoplastic stroma that inhibits the entry of chemotherapy drugs. Therefore an intermediate strategy that modulates the stroma could be a potential therapeutic option. Studies have shown effects on cell proliferation, migration and cytokines with of Low Intensity Ultrasounds (LIUS). The use of LIUS as a novel, non-invasive tool to modulate the stroma was investigated in pancreatic cancer models.

Methods: Pancreatic tumor cell lines, Panc-1 and MiaPaCa and the fibroblast cell line BJ-hTERT were exposed to LIUS (1035kHz and 10V) twice per day over a 3 day period. Cellular proliferation, viability and vitality were assessed using the MTT and Alamar Blue assays. Furthermore, mRNA expression of epithelial (EpCAM, CK and MUC1), mesenchymal (SNAIL and ZEB) and angiogenesis (VEGF) markers were assessed in tumor cells and classic activation markers were assessed in fibroblasts (a-SMA, FAP and FSP) via qPCR. Cell migration in response to LIUS was also assessed over a 23 hour period using a wound healing assay and assessment of cell confluence using the PAULA microscope.

Results: Small differences were observed in tumor cell viability after exposure to LIUS, whereas a reduction in cell vitality was observed, particularly in MiaPaCa cells. Furthermore, an increase in the expression of the epithelial marker EpCAM was observed in tumors cells and a reduced expression of the tumor marker MUC1 was observed in Panc-1 cells. There was also a small but significant decrease in the overall levels of VEGF mRNA expression. Modest differences were also observed in the viability of BJ-hTERT fibroblasts after exposure to LIUS and there was a significant reduction in the expression of the fibroblast activation factor FAP. There was a reduction in the migration capacity of Panc-1 cells after exposure to LIUS (46% wound closure LIUS vs. 17% no LIUS). BJ-hTERT usually close the wound within 12-18 hours. However, 23 hours after LIUS exposure the wound had not closed. The effect of LIUS appears to be dose dependent as between 24-48 hours after LIUS exposure, the migration capacity of the cells was restored.

Conclusions: The effects of LIUS on cell proliferation and viability and gene expression are small and may not be biologically relevant. However, the effects of LIUS on cell migration are more profound, particularly in fibroblast cells. Low Intensity Ultrasounds (LIUS) could be a complementary tool in the treatment of pancreatic cancer that targets the stroma and inhibits tumor dissemination.

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