

# The effects of LIPUS on ctDNA release in the medium of NSCLC cell lines.

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**Background:** Low Intensity Pulsed Ultrasound (LIPUS) application has been shown to have an encouraging effect in inducing a transient pore formation through cellular membranes. This permeability condition has been demonstrated to be useful in enhancing gene and drug delivery. Nowadays, in the management of NSCLC patients, the use of liquid biopsy has entered the clinical practice. One of the main limits in the analysis of circulating tumor DNA is the low concentration rate of nucleic acids in body fluids. Ultrasound stimulation (US) has been recently demonstrated to be effective for the release of specific circulating tumor biomarkers in many mouse models. We demonstrated the role of US in inducing the release of tumor DNA fragments (rtDNA) in NSCLC without inducing any apoptotic or necrotic event.

**Material and Methods:** EGFR wt and del19 NSCLC cells (A549, HCC827) were cultured in RPMI1640 with 10% FBS, 1% pen/strep at 37 °C and 5% CO<sub>2</sub>. The day before the sonication cells were seeded in a 24-well plates (20.000 cells/well HCC827; 10.000 cells/well A549). Each well was exposed at the following sonication protocol: frequencies (650 kHz, 1 MHz); acoustic pressure (250 kPa, 25 kPa); 25% duty cycle with three different exposure time points (1, 3, 7.5 min). After US treatment, the cells were incubated for 24h and then cell viability was performed by Cell Titer-Glo® Luminescent Cell Viability Assay. Each experiment was performed in triplicate.

**Results:** NSCLC cells have been subjected to sonoporation at different exposure time points as well as ultrasonic acoustic pressures and frequencies. We evaluated the viability cells to exclude the possibility that rtDNA analysis could be affected by apoptotic or necrotic DNA fractions. In fact, after ultrasound exposure no significant reduction of cell viability, in terms of ATP content, has been shown. Moreover, the analysis of DNA fragments content, released in the medium, showed a different behaviour on the basis of EGFR mutational status. Indeed, in EGFR mutated cells the concentration of rtDNA was significantly higher than control cells after performing sonoporation at

250 kPa and 1 MHz . For the EGFR wild type cell line, no variation of ctDNA at different exposure time points and pressure has been shown.

**Conclusions:** the assessment of ctDNA is strongly influenced by its amount. Therefore, US application to enhance the release, is of great interest not only in NSCLC but also for all the "oncogene addicted" cancers.