Low-intensity ultrasounds improve nanoparticles-complexes delivery in cancer cells in vitro: new insights for more efficient therapeutic strategies sparing normal cells

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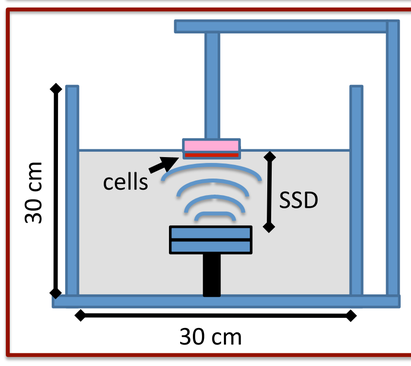
Background

The possibility to combine Low Intensity UltraSound (LIUS) and Nanoparticles (NP) could represent a promising strategy for drugs delivery in tumors difficult to treat overcoming resistance to therapies. On one side the NP can carry drugs that specifically target the tumors on the other the LIUS can facilitate and direct the delivery to the tumor cells. In this study, we investigated whether LIUS, at intensities lower than 120mW/cm^2, could constitute a novel approach to improve NP delivery to tumor cells.

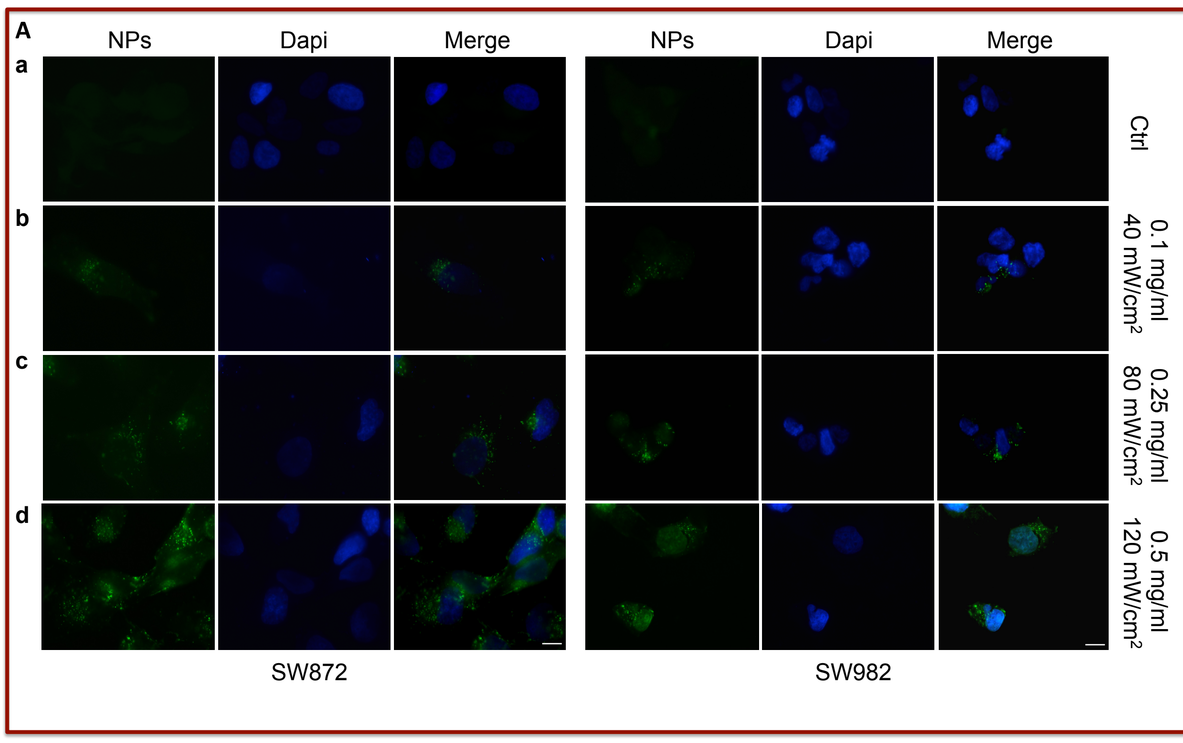
Methods

LIUS at different intensities and exposure time were applied to tumor and normal cells to evaluate efficiency in uptake of labeled human ferritin (HFt)-based NP, in delivery of NP complexed Firefly luciferase reported gene (lipoplex-LUC), and tumor-killing by chemotherapeutic agent.

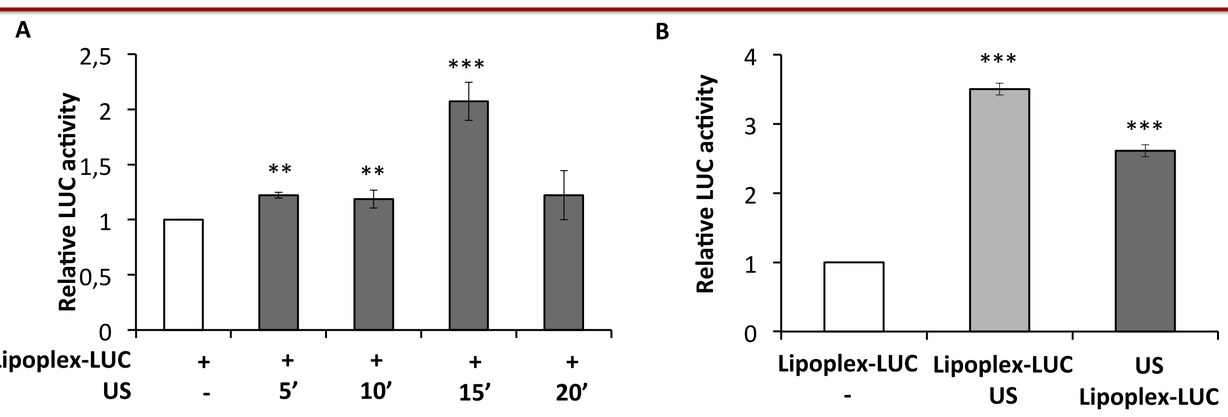
Results



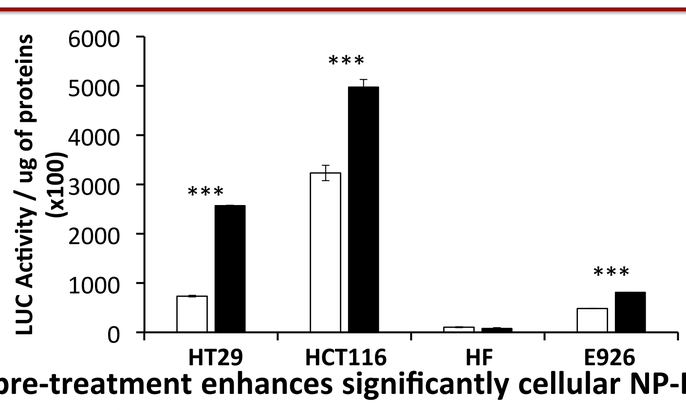
1. Ultrasounds setup. For US exposure, a line of ultrasonic signal was generated by a piezoelectric unfocussed transducer immersed at the bottom of a tank filled with degassed water, powered to a signal generator and a signal amplifier. A sinusoidal signal at the frequency of 1 MHz was generated and measured by a needle hydrophone of 0.5 mm diameter with a sensitivity of 483 mV/MPa at 1 MHz, connected to an oscilloscope. Continuous ultrasound exposures in terms of 'spatial peak temporal average intensity' equal to 40, 80, 120 and 150 mW/cm^2 were administered on a petri dish (60 mm), submerged up to half of its thickness and aligned coaxially with the transducer, at a fixed distance Source-dish Surface Distance (SSD) from the transducer.



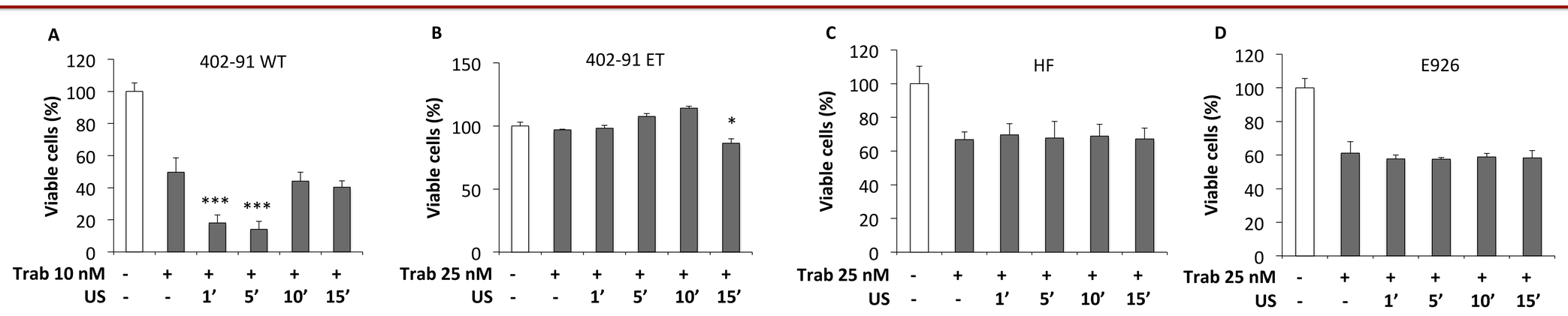
2. US treatments increases HFt-based NPs uptake. (A) SW872 and SW982 cells were plated on poly-l lysine coated slides and the day after incubated for 45 min in the presence of fluorescein-labelled HFt-based NPs at different concentration (0.1 – 0.25 -0.5 mg/ml). Thus, cells were exposed 15 minutes to ultrasound source with increasing intensities (40 - 80 - 120 mW/cm^2). Control cells were treated 1 h with HFt-based NPs alone. The cells were counterstained with Hoechst to highlight nuclei and analyzed for immunofluorescence dots. Fluorescence dots were quantified as fraction of pixels over the cutoff of 10 using in the green channel of each image. Scale bar is 10 µm. (B) Histogram reported percentage of uptake and error bars represent the standard deviation of the measured fractions in the images for each experimental condition.



3. US exposure enhances cellular uptake of LipoplexLUC NP complexes.
A. HT29 cells were delivered with lipoplex-LUC complexes and right after US treated at indicated times lengths. **B.** HT29 cells plated as reported in A, then cells were delivered with lipoplex-LUC complexes, right before or after US treatments. In A and B cells were collected 48 h after treatments. DNA delivery efficiency was assessed by luciferase assays and values were normalized to protein content and relative LUC activity quantified with respect to control set to 1.0. Each experiments have been repeated three times in triplicate, means and standard deviation of representative experiments are reported.



4. Ultrasound pre-treatment enhances significantly cellular NP-DNA complexes uptake in cancer cells but not normal cells. Cancer HT29, HCT116 and normal HF, E926 cells were plated and then 24 hours later, growth media was replaced with OPTIMEM, delivered with liploplex-LUC complexes and thereafter ultrasounds treated for the indicated times. Cells were collected 48 h after treatments. DNA transduction efficiency was evaluated by luciferase assays and values were normalized to protein content and relative LUC activity quantified respect to control set to 1.0. Each experiments have been repeated three times in triplicate, means and standard deviation of representative experiments are reported.



5. US treatments increases trabectedin uptake in tumor but not in normal cell.
(A, B, C and D, respectively) 402-91 WT, 402-91 ET, HF and E926 cells were plated at density of 150000 cells in 60 mm dish, and the day after treated for 1h with trabectedin at a concentration of 10 or 25 nM. During the treatment cells were also exposed 1, 5, 10 or 15 minutes to LIUS source at intensities of 80 mW/cm^2. Cell vitality was evaluated by Crystal violet assay 48h after drug removal. Histogram reported percentage of viable cells and error bars represent the standard deviation.

Conclusions

Our data shed novel lights on the potential application of LIUS for the design and development of novel therapeutic strategies aiming to efficiently deliver of NP loaded cargos or anticancer drugs into more aggressive and unresponsive tumors niche.