Induction of apoptosis by high levels of oscillatory shear strain: proof of concept in a human colon cancer metastasis cell line.

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Purpose / Introduction: Mechanotransduction is a biological process enabling cells to convert mechanical cues into various biological responses[1]. The survival of many cell types is actually conditioned by the mechanical status of their environment, which may balance pro and anti apoptotic effects[2,3]. In this context, it is more likely that cells respond to shear stress rather than to compressional forces due to the incompressible nature of tissue. Thus, it is theoretically thinkable to selectively induce cellular death by the controlled application of shear strain/stress. Unlike e.g. HIFU (high intensity focused ultrasounds), here the energy deposited locally by the mechanical strains/stresses would by far not be sufficient to lead to any destruction of cellular membranes but is rather used to trigger specific signals within the cell. We establish the proof of concept for this proclaimed effect in a cell line of colon cancer metastasis. Estimation of the applied shear strain levels required for this effect were obtained via MR strain imaging of thin gel slices.

Subjects and Methods: DHDK12 colon cancer metastasis cells were grown in monolayers on the surface of cell culture plates (diameter: 3.6cm) using Ham's F-10 medium. At confluency, one plate was subjected for 4 hrs to a 90Hz uniaxial sinusoidal motion parallel to the cell layer by a rigidly linked modal exciter. One control plate was left without any vibration. The strain levels induced in the cell monolayer were measured on a control cell culture plate loaded with a thin layer of agarose gel (1mm, 2g/l) and covered with liquid to simulate the medium. Shear strain images were obtained on a 7T Bruker system using a spin echo MR elastography sequence synchronized with a 90Hz vibration of the imaged plate (TE/TR = 38.9/1744 ms) with 4 wave offsets, 3 encoding directions and a 300 $(\mu m)^3$ isotropic resolution. Strain was evaluated as the maximum shear strain, i.e. the difference between the maximum and the minimum eigenvalues of the strain tensor. At the end of the experiment, cells were detached from the plate, colored with trypan blue and a count of colored (i.e. injured cells) or translucent (i.e. "healthy") cells was made with a hematocytometer. Levels of caspase-3, an effector caspase downstream of the apoptosis pathway, were assessed by western blotting, using β-actin as the loading marker.

Results: This particular setup yielded maximum shear strains on the order of 5.5 ± 1.1 % in the first 300µm immediately above the plastic surface (Fig.1). Mind, that typical maximum shear strain levels for in-vivo MR-elastography experiments do not exceed 0.1%! As expected, shear levels were negligible in the liquid medium above the thin layer of solid medium. Vibrations were sufficient to induce cell death, as observed by the 4-5 fold increase in the count of dead cells after vibrations (Fig. 2). Before vibrations, 2.9±1.5% (5.3±2.6%) dead cells were found in the two plates, respectively. After 4 hours, 4.5±1.4% dead cells were found in the control plate, vs. 22.3±3.9 % in the plate having undergone vibration. Caspase-3 levels were elevated in the cells having undergone vibrations, indicating that an activation of the apoptosis pathway was involved in the observed increase in cell deaths.

Discussion/Conclusion: We show that the application of 5% maximum shear strain at 90Hz for 4 hours has the ability to induce apoptosis in vitro on a human cell line of cancer metastasis. This proof of concept indicates that the selective application of high strain shear waves of low frequency may become a viable alternative for tumor therapy.

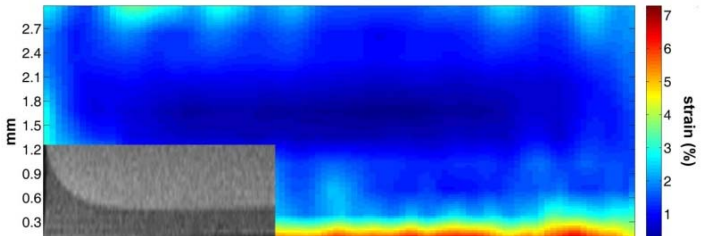

Fig. 1: Shear strain map (side view) obtained with the control agarose cell culture plate (color scale in %).The image resolution was up-sampled in the vertical dimension. The thin layer of gel is visible in the strain map close to the surface (bottom).The T2-weighted image (overlaid on the lower left corner of the image) depicts the localization of the gel layer as a region with lower intensity due to the shorter T2 of agaorse gel compared to liquid medium.

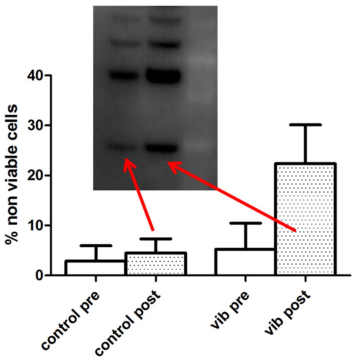

Fig. 2: Cell count (expressed as percent of nonviable cells) for the control plate (left) vs. the vibrated plate (right) before (white bars) or after (dashed bars) 4 hours of 5% oscillatory strain at 90Hz. Insert: western blot of caspase-3. An elevated level of caspase-3 was observed in the cells having undergone mechanical shear, indicating an involvement of the apoptotic pathway.

[1] Ingber, FASEB 2006 20(7)
[2] Jaalouk & al., Nat Rev Mol Cell Biol, 2009 10(1)
[3] Cheng & al., PLOS One, 2009 4(2)