Ultrasound-induced mechanical strains stimulate cell division pathway in vivo

L.Zamfirov[1], E.Teston[1], K.Sollier[2], ME.Fernandez-Sanchez[2], C.Demené[1], E.Farge[2], M.Tanter[1]
[1] Physique des ondes pour la médecine, Inserm U1273, ESPCI Paris, CNRS, PSL Research University, Paris, France,
[2] Institut Curie, Centre de Recherche, PSL Research University, CNRS UMR 168, Physicochimie Curie Mechanics and Genetics of Embryonic and Tumour Development, INSERM, Paris, France.

Background, Motivation and Objective

Recently, cell proliferation has been shown to be modulated by local mechanical stress, through the activation of the mechanotransductive Ret/β-catenin pathway in colonic crypt cells [Avvisato et al, 2007, J. Cell Science; Fernandez-Sanchez et al, 2015, Nature]. Activation of this signaling pathway leads to cellular division mechanisms amplification.
In this context, we assessed the effect of ultrasound stimulation on this mechanotransductive pathway activation, through the quantification of Ret kinase phosphorylation. Here, ultrasound is used both to remotely induce and monitor controlled mechanical deformations of tissues.

Statement of Contribution/Methods

Mechanical stress was induced via radiation force with a L7-4 probe (128 elements, 0.298mm pitch, 5.5MHz) driven by a programmable ultrasound system (Verasonics). This radiation force was remotely generated with series of 250-µs (1250 cycles) focused beam at PRF of 4Hz distributed at several locations and depths to cover the colonic tissue extent for 1h. For each focused beam, ultrafast imaging (1 cycle, PRF 10kHz) was used to image local tissue strain and relaxation.
Ex vivo experiments were performed on wild-type mice distal colon explants. Organs were incubated at 37°C in oxygenated Krebs solution. *In vivo* experiments were achieved in anesthetized wild type mice. After stimulation, colons were fixed, frozen and cryosectioned. Labeling was carried out to show Ret kinase activation, as it is the primary mechanical sensor of the signaling pathway.

Results/Discussion

Acoustic radiation force beams typically induced a 10 µm displacement, representing at the focal spot (0.3 and 2.1 mm for respective lateral and axial focal size) a $8.3.10^{-6}$ N force (i.e. a $4.4.10^{4}$ $N.m^{-3}$ volumetric force). Both *ex vivo* and *in vivo* results show that ultrasound-mediated mechanical stress significantly increases Ret phosphorylation in colonic crypts in stimulated mice compared to control mice (n=6). The number of phosphoRet-positive crypts were significantly increased by 1.6-fold in treated mice (17.3% versus 27.6% and 23.2% versus 39.5%, respectively for *ex vivo* and *in vivo* experiments, $p<0.01$).

This activation is consistent with the rapid dynamics associated with mechanotransduction processes [Na et al, 2008, PNAS] and a direct mechanical activation of Ret. Ret phosphorylation is required upstream of the mechanosensitive Ret/β-catenin pathway activation, which is implied in cell proliferation, as demonstrated in [Fernandez-Sanchez et al, 2015, Nature]. These data suggest that ultrasound is a powerful tool to remotely induce mechanotransduction in biological systems. It is the first step for deciphering ultrasound-induced β-catenin pathway activation and towards an extensive analysis of promoting cell division *in vivo* by acoustic force.