Tumor stiffening, a key determinant of tumor progression, is reversed by photohyperthermia: *In vivo* monitoring by ultrasound elastography

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Tumor stiffening, which stems from aberrant production of extracellular matrix and in particular from collagen fiber network, has been considered as a predictive marker of tumor malignancy. Moreover experimental evidences suggest that tumor rigidity and altered mechanics are, per se, key modulators of tumor progression. Our group has recently shown that thermal therapy denatures tumor stroma, directly impacting on tumor permeation properties and drug/nanoparticles penetration[1]. Herein we aimed to evaluate the therapeutic efficacy of the photothermal therapy (PTT) mediated by carbon nanotubes (CNTs) by correlating tumor growth, stroma integrity and mechanical properties. At the microscopic level, PTT impact on tumor stroma was characterized by second-harmonic generation (SHG) imaging microscopy. At the macroscopic level, ultrasound elastography was used to assess mechanical stiffness of the tumor tissue during the PTT treatment and over 10 days after.

PTT was tested *in vivo* on epidermoid carcinoma xenografts implanted in mice via two distinct types of heating protocols: moderate hyperthermia (43°C - 45°C for 20 min, repeated twice) and thermal ablation (50°C for 3 min, repeated twice). We aimed to determine the best compromise between overheating limitation, thermal damage and outcome on tumor regression.

Tumor stiffness was monitored throughout the period of the treatment using shear wave elastography. While non-treated tumors show increasing stiffness over time, both photothermal treatments reversed tumor stiffening, while diminishing tumor volume. Such macroscopic features correlated to microscopic damages of the tumor stroma induced by local heating of CNTs. Specific visualization of CNTs distribution on tumor slices shows the colocalization of CNTs with thermal damages on collagen fibers (visualized by SHG) and adjacent cells (visualized by biphoton autofluorescence). This highlights that carbon nanotube heating induces a local destructuration of the extracellular matrix and contributes to the decrease of the global tumor stiffness.

This study highlights the potential of ultrasonic shear wave elastography for monitoring the effects of hyperthermia on tumor tissues via the evolution of their mechanic properties, in correlation with stroma integrity. Moreover it enlarge the use of ultrasonic elastography as a tool for non-invasive personalized monitoring method for tumor therapy. Reversion of tumor stiffening by photothermal treatment could be a promising adjuvant therapy for controlling tumor progression.

1. Kolosnjaj-Tabi *et al*, Heat-Generating Iron Oxide Nanocubes: Subtle “Destructurators” of the Tumoral Microenvironment. ACS Nano 2014 8 (5), 4268-4283