MR-Elastography, a new biomarker of the tumor vascularization in a colon cancer mice model

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Introduction

Assessment and follow-up of neo-angiogenesis are major challenges to characterize the malignancy of tumors and to test the efficacy of novel treatments. Here, Magnetic Resonance Elastography (MRE) has a vast potential to provide new biomarkers characterizing tumors in animal models (1). In our work, we aim at studying the alterations of the vascularization during the spontaneous growth of the tumor and after a treatment with an antivascular agent (combretastatin A4 phosphate CA4P)(2,3). Our working hypothesis is that alteration of the vasculature will induce significant changes in the viscoelastic properties of tissue.

Material & Methods

Balb-C mice were used with a colorectal cancer model (CT26) implanted either subcutaneously into the flank (ectotopic model) or on the cecum (orthotopic model). First, the spontaneous growth of the tumor was followed at day 5 (primitive stage), day 11 (angiogenic stage), day 14 and 18 (late stage). Secondly, mice were treated at day 10 with 100mg/Kg of CA4P and MRI/MRE was performed 24h after injection. Studies were performed in-vivo in a horizontal 7T scanner (Bruker, Pharmascan). We recorded for each animal high resolution T2-weighted images (RARE sequence, 125µm x 125µm in plane resolution), 3D steady-state MR-Elastography images with a vibration frequency of 1000 Hz (250µm isotropic image resolution) and Diffusion MR images (b-values: 250, 500, 750, 1000 and 2000 s/mm^{-2}). After MRI, the tumors were excised and the endothelial cells were marked with CD31 as well as the proliferative cells with KI67 for detailed histological analysis.

Results

Histology (Fig.1) showed that the blood vessel density (BVD) (full lines) increased significantly ($P<0.01$) between the primitive stage at day 5 and the angiogenic stage at day 11 for the two implantations. After antivascular treatment, a significant decrease ($P<0.05$) of the BVD in the viable rim was measured in the ectotopic model (see blue arrow in Fig.1) and not in the orthotopic model (red arrow).

Regarding the cellularity (Fig. 1, dotted lines), only the ectotopic model and not the orthotopic model showed a significant increase ($P<0.01$) between primitive and angiogenic stage. The alterations in cellularity for the two tumor models were properly reflected by changes in ADC: the ADC dropped significantly for the ectotopic model between primitive and angiogenic stage ($0.66+/-0.06\ 10^{-3}$ mm^2/s versus $0.45+/-0.04\ 10^{-3}$ mm^2/s, $P<0.05$) and correlated well to cellularity ($r=0.85$, $p=0.02$) whereas no significant changes ADC were observed for the orthotopic model ($0.60+/-0.08\ 10^{-3}$ mm^2/s versus $0.56+/-0.04\ 10^{-3}$ mm^2/s). After injection of CA4P, the ADC increased significantly ($0.65+/-0.15\ 10^{-3}$ mm^2/s in ectotopic model and $0.78+/-0.12\ 10^{-3}$ mm^2/s in orthotopic model) which could be attributed to cell death and to a decrease of cellularity.

Fig.2 shows the evolution of the elasticity (Gd) as a function of BVD (with a similar evolution for the viscosity Gl, not shown). Each stage of the tumor development is represented by its average value for Gd and BVD, hence one filled circle for each tumor model indicating the primitive stage and 3 filled circles (days 11, 14, and 18) indicating the angiogenic stage. The measurements of Gd and Gl show a significant increase of the mechanical properties when the BVD increased.

In other words, when the tumor switched from the primitive to the angiogenic stage, the evolution of the two mechanical parameters was significantly modified ($P<0.001$) This behavior was common to both tumor models, in contrast to what was observed for the ADC. After treatment, only the ectotopic model demonstrated significant changes in Gd and Gl (see blue arrow in Fig.2) while the viscoelastic properties of the orthotopic model did not change significantly (red arrow in Fig.2). This very different behavior correlated well to the differences observed for the BVD under treatment: while the BVD for the ectotopic model returned almost back to the primitive stage (and likewise the elasticity), the BVD for the orthotopic model did not change (and likewise the elasticity). To summarize, an increase of the vascularization correlated with an increase of the mechanical properties and a reduction of the vascularization induced by an antivascular treatment lead to a decrease of Gd and Gl.

Fig 1: Evolution of the number of vessels (mm^{-2}) and of the cellularity (mm^{-2})

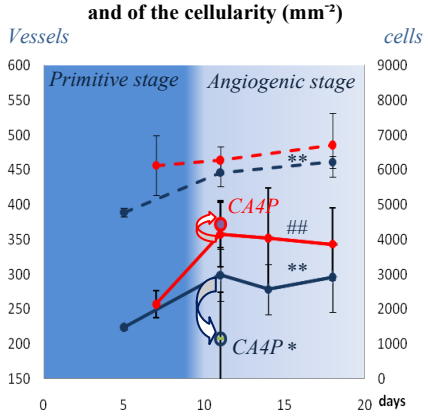
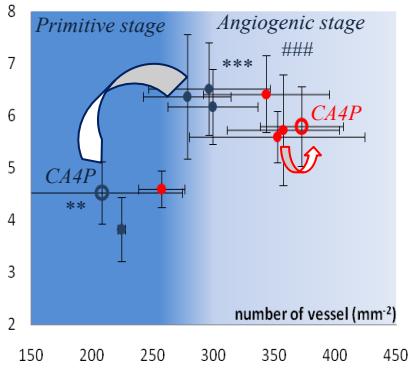


Fig 2: Evolution of the Elasticity Gd (kPa)



red : orthotopic model (statistically significant difference: #)blue: ectotopic model(statistically significant difference :)*

Discussion & Conclusion

Our results show that it was possible to follow the evolution of a tumor and the efficacy of an antivascular agent using MRE and that MRE performed better than Diffusion MRI in this regard. It was established that the switch between primitive stage and angiogenic stage for both models could be followed by MRE. Although several alterations of the structure (vascularization, cellularity and extracellular components) could induce alterations of the mechanical properties, there was a close correlation between the vascularization and the mechanical properties. Finally, the viscosity and the elasticity are potentially new biomarkers to trace alterations of the vascularization induced by antivascular treatments.

References

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