The role of vessel maturation and vessel functionality in spontaneous fluctuations of T2*-weighted GRE signal within tumors

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Introduction: Acute hypoxia (transient cycles of hypoxia-reoxygenation) is known to occur in solid tumors and is generally believed to be caused by tumor blood flow instabilities. We recently demonstrated that T2* weighted gradient echo (T2*w GRE) MRI is a powerful non-invasive method for investigating periodic changes in tumor pO_2 and blood flow associated with acute hypoxia (Baudelet et al, *Phys Med Bio*l 2004; 49:3389). Here, we investigated the possible correlation between tumor vessel immaturity, vessel functionality and T2*w GRE signal fluctuations.

Methods: Intramuscularly implanted FSa II fibrosarcoma-bearing mice were imaged at 4.7T. A surface coil, 2.5 cm in diameter, was used for RF transmission and reception. A single 1.3 mm thick slice was localized such that it passed through the tumor center. Maps of spontaneous fluctuations of MR signal intensity in tumor tissue during air breathing were obtained using a T2*-weighted GRE sequence (TR=200 ms, TE=18 ms, flip angle=45°, 12.5 kHz receiver bandwidth, 64 phase and frequency encode steps, linear encoding order, 3 cm FOV, 2 averages, 25.6 sec/image, runs of 140 sequential images). This same sequence was also employed during air-5% CO2 breathing (hypercapnia) and carbogen breathing (hypercapnic hyperoxia) to obtain parametric maps representing vessel maturation and vessel function, respectively. Vascular density, vessel maturation and vessel perfusion were also assessed histologically by using CD31 labeling, alpha-Smooth Muscle Actin immunoreactivity, and Hoechst 33242 labeling, respectively.

Results: About 50% of the tumor fluctuations occurred in functional tumor regions (responsive to carbogen), and 80% occurred in tumor regions with immature vessels (lack of response to hypercapnia). The proportion of hypercapnia responsive voxels were found to be twice greater in fluctuating than in non-fluctuating tumor areas (p: 0.22 vs 0.13). Similarly, the proportion of functional voxels was somewhat greater in fluctuating tumor areas (p: 0.54 vs 0.43). The mean values of MR signal changes during hypercapnia (VD) and during carbogen breathing (VF) (significant voxels only) were also larger in fluctuating tumor areas than in non fluctuating ones ($p<.05$). Histological results showed that fluctuating areas were located mostly in perfused tumor areas. In these perfused areas, there was not a high incidence of α-SMA immunoreactivity. The few regions with positively-staining α-SMA immunoreactivity (situated primarily in the periphery for this particular tumor), however, presented a high incidence of signal fluctuations (see fig.2). Thus, although most of the signal fluctuations occurred in regions with immature vessels, the presence of mature vessels did not preclude the occurrence of signal fluctuations.

Discussion: Adequate vessel functionality and advanced vessel maturation are not crucial factors involved in spontaneous T2*w GRE signal fluctuations, since a large fraction of fluctuations were observed to occur in non-perfused and/or immature vessels.

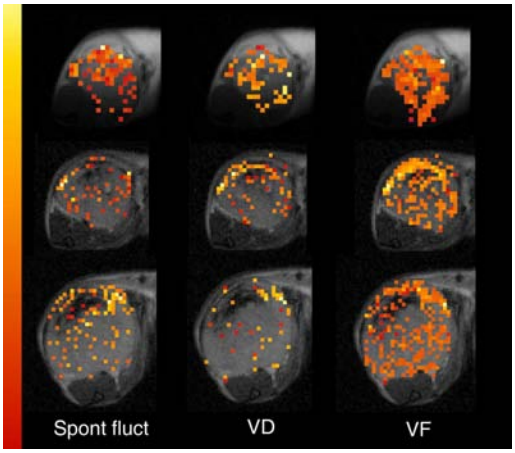

Fig. 1 (left) Coregistration of spontaneous fluctuations, vascular maturation and vascular function maps for 3 separate tumors in mice.

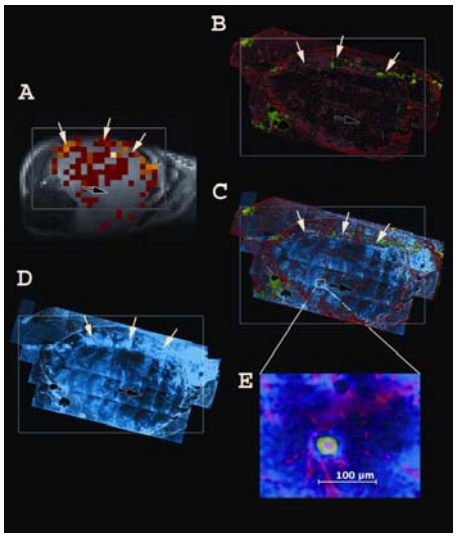

Fig. 2 (right) Comparison of MRI with histology. A: Map of spontaneous T2*-w GRE SI fluctuations. *B:* Double staining for CD31-immunoreactive endothelial cell (red) and α-SMA-immunoreactivity (green).
C: Triple staining for CD31 (red), α-SMA (green) and Hoechst 33342 (blue).
D: Hoechst 33342 labeling assay.
E: Enlargement of a tumor area showing an arteriole surrounded with α-SMA-positive cells (green).