Investigating tumour cycling hypoxia with resting state MRI: relationship with systemic changes and influence of noise

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Target audience: Researchers interested in cancer studies, particularly cycling tumour hypoxia.

Introduction: Solid tumours have been observed to exhibit regions of transient, cycling hypoxia and subsequent reoxygenation due to spontaneous fluctuations in blood flow and oxygenation [1, 2]. This has been shown to contribute to resistance to chemotherapy [3] and radiotherapy [4], as well as tumour progression and development of metastatic disease [5, 6]. $R_{\text{r}}^*$ (=1/T2*) estimates from gradient echo MRI (GRE-MRI) have been employed in the study of tumour cycling hypoxia due to its dependence on deoxygenated hemoglobin [1, 7]. However, the influence of systemic changes in blood oxygenation on tumour $R_{\text{r}}^*$ fluctuations has not been directly investigated, particularly in anaesthetised animals, in which both properties can vary. In this study, we have modelled systemic arterial ($R_{\text{a}}^*$) and tumour vasculature as shown in Fig. 1, in which the tumour is influenced by systemic changes in oxygenation, but can also modify this parameter. We have measured resting state $R_{\text{r}}^*$ fluctuations in the tumour with simultaneous systemic blood $O_2$ saturation, in order to assess their relationship.

Furthermore, we estimated the influence of measurement noise on tumour $R_{\text{r}}^*$ fluctuations.

Methods: 5x10^6 SW1222 (n=6) or LS174T (n=5) colorectal carcinoma cells were injected subcutaneously into MIF1 nu/nu mice. After growing to an approximate volume of 500mm$^3$, tumours were imaged on a 9.4T Agilent VNMRS 20cm horizontal-bore system, with a 39mm birdcage coil, using a multi-slice, multi-gradient echo (GEMS) sequence. Mice were anaesthetised using isoflurane (1.25% in medical air). Respiratory frequency varied between 43-92 breaths/min and temperature was maintained at 36.7 °C. A scan of 60min duration was performed to evaluate spontaneous fluctuations in tumour $R_{\text{r}}^*$ (=1/T2*). Simultaneously, arterial hemoglobin $O_2$ saturation ($O_{\text{sat}}$) was measured by pulse oximetry on the thigh (MouseOx, Starr Life Sciences Corp, Oakmont, PA). Voxel-wise post-processing included: i) Resting state $R_{\text{SD}}$–RS$_{\text{SD}}$ maps, that depict the standard deviation of unchallenged $R_{\text{r}}^*$ time courses in each voxel. ii) $R_{\text{SD}}$/uncertainty maps represent voxels whose $R_{\text{r}}^*$ amplitude changes are above the uncertainty due to background noise, which was estimated using a Markov Chain Monte Carlo (MCMC) approach [8]. iii) p-value maps show the Pearson’s correlation significance between $R_{\text{r}}^*$ time courses and $O_{\text{sat}}$ saturation ($O_{\text{sat}}, R_{\text{r}}^*$). GEMS parameters: SPGR sequence with TR=59.62ms, 5 echoes, TE=2ms, echo spacing=2ms, 5 slices, 64x64 matrix, voxel volume 312x312x1500μm, FA=20°.

Results: We present example maps of i) Resting State($R_{\text{SD}}$), ii) $R_{\text{SD}}$/uncertainty and iii) $p(O_{\text{sat}}, R_{\text{r}}^*)$, as well as arterial $O_2$saturation and averaged $\Delta R_{\text{r}}^*$ timecourses. Resting State($R_{\text{SD}}$) maps show clear variations in $R_{\text{r}}^*$ (Fig. 2a). Maps of the ratio of the variance in temporal $R_{\text{r}}^*$ fluctuations and measurement uncertainty (from MCMC analysis) are shown in Fig. 2b, in which voxels with $R_{\text{SD}}$/uncertainty > 1 indicate regions within the tumour with temporal fluctuations greater than measurement noise level (percentage of voxels above noise level was 50.3% and 71.1% for SW1222 and LS174T tumours, respectively). Fig. 2c shows the p-values obtained from the correlation between tumour arterial hemoglobin $O_2$saturation (depicted in Fig. 2d) and individual voxel $R_{\text{r}}^*$ timecourses. We observed clusters of voxels with $R_{\text{r}}^*$ significantly correlated ($p < 0.01$) with systemic $O_2$ saturation (arrow), and Fig. 2e shows the averaged $\Delta R_{\text{r}}^*$ for the voxels with $p(O_{\text{sat}}, R_{\text{r}}^*) < 0.01$, showing a clear correspondence with the $O_{\text{sat}}$ curve. However, other regions with significant $R_{\text{r}}^*$ fluctuations showed no correlation with systemic $O_{\text{sat}}$, and were consequently categorised as exhibiting tumour-specific fluctuations. According to this analysis, the mean percentage of tumour voxels displaying tumour-specific fluctuations was 38.3% and 37.5% for SW1222 and LS174T tumours, respectively ($p=0.18$, Mann-Whitney U test); the percentage of voxels displaying systemic only $R_{\text{r}}^*$ fluctuations was 17.5% and 15.2% for SW1222 and LS174T, respectively ($p=0.93$, Mann-Whitney U test) (Fig. 3).

Discussion & Conclusion: Maps of resting state $R_{\text{r}}^*$ fluctuations included tumour voxels in which the fluctuations were greater than the uncertainty associated with measurement noise. Within these regions, it was found that $R_{\text{SD}}$ maps show regions of high $R_{\text{r}}^*$ amplitude variations that correlate significantly with systemic changes in blood oxygenation. However, we also observed fluctuations that were not significantly correlated with systemic oxygenation changes, which were considered to be related to localised (tumour-specific) changes in oxygenation. One limitation of this method is its inability to detect systemic effects in pixels displaying a combination of systemic and tumour-specific effects. This limitation could potentially be addressed using independent component analysis techniques. LS174T tumours displayed a higher percentage of tumour-specific fluctuating voxels than SW1222 tumours, although this difference was not statistically significant. We also found no difference between tumour types when analysing the percentage of systemic fluctuating voxels. These results suggest that, even though LS174T tumours are less vascularised and less perfused than SW1222 tumours [9], they show no differences with regard to $R_{\text{r}}^*$ spontaneous fluctuations.

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