

Imaging biomarkers of cell death: a comparison between viscoelasticity and ADC in an orthotopic breast cancer xenograft model

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Introduction:

There is a continuing need to identify and evaluate informative functional imaging biomarkers in oncology. Magnetic resonance elastography (MRE) is an emerging imaging technique being increasingly exploited for the assessment and visualization of tissue mechanical properties *in vivo*. Recent pre-clinical investigations have reported significant reductions in tumour viscosity and elasticity following treatment with conventional chemotherapy or vascular disrupting agents, associated with a pathologically-confirmed reduction in cell proliferation and increased necrosis [1-3]. Quantitation of the tumour apparent diffusion coefficient (ADC), measured by diffusion-weighted imaging (DWI), is also being actively evaluated as an oncology imaging biomarker [4, 5]. The reduction of density and cell membrane integrity associated with treatment-induced tumour necrosis typically results in an increase in ADC. In evaluating MRE for the provision of novel imaging biomarkers, both pathological validation and correlation with more established imaging biomarkers is necessary [6]. To this end, we have compared the relationship between change of MRE and DWI and change of orthotopically propagated BT474 breast cancer xenografts pathology along tumour progression and/or following treatment with the novel AGC kinase inhibitor AT13148, previously shown to induce a strong apoptotic phenotype in this model [7].

Methods:

All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986. BT474 breast carcinoma cells (5×10^6) were injected subcutaneously in the mammary fat pad of female NCr nude mice. MRE data were acquired from anaesthetised mice bearing size-matched tumours prior to and 24 hours after treatment with either 40mg/kg AT13148 p.o. (n=6) or vehicle alone (n=6). 3D MRE data were acquired with a 7 Tesla MicroImaging horizontal MRI system (Bruker Instruments, Ettlingen, Germany), using a spin-echo sequence (TE/TR = 27/1001ms, FOV=15.8mm×15.8mm) modified with sinusoidal motion-sensitizing gradients, synchronized with a continuous 1 kHz sinusoidal wave generated by an electromagnetic shaker (Brüel & Kjaer, Naerum, Denmark), applied directly through a carbon fibre rod to a square piston positioned onto the tumour. MRE data were acquired from ten 0.3mm thick axial slices through the centre of the tumour. Care was taken in positioning the animal so as to acquire MRE data from the same location in the tumour prior to and post-treatment. Parametric maps of the complex shear modulus $|G^*|$, elasticity G_d , viscosity G_v , shear wave attenuation α , and shear wave speed c_s were reconstructed with an isotropic pixel size of 300 μ m, and the mean value determined from a region of interest covering the whole tumour from the central two slices. Diffusion-weighted images (TE/TR=32/1500ms, b values=200, 300, 500, 750, 1000 s/mm², three 1mm thick slices) were also acquired for determination of tumour ADC. Following the post-treatment scan, tumours were excised, formalin fixed, and paraffin-embedded sections cut and stained with haematoxylin and eosin (H&E) and picrosirius red for the histological assessment of necrosis and collagen respectively.

Results:

The hyperintense signal (hyper-signal) area in T₂-weighted images was associated with a localised region of dying cells, with the pattern of cell shrinkage, cell-cell membrane detachment and maintenance of collagen in the extracellular matrix revealed by histology (Fig. 1). Marked intra-tumoural heterogeneity in T₂-weighted images was apparent prior to and post-treatment, and an increase in the hyper-signal area within the tumour core over 24 hours, irrespective of treatment. The spatial distribution of $|G^*|$ and ADC values were closely associated with the overall T₂-weighted image contrast, with reduced $|G^*|$ and elevated ADC values within the T₂-weighted hyper-signal area. Different signal level was conspicuous in both $|G^*|$ and ADC maps between the T₂-weighted hyper-signal area and iso-signal area (Fig. 1). Changes in $|G^*|$, G_d , G_v and c_s (except for α) and ADC for each tumour over 24 hours were significantly correlated with the change of percentage hyper-signal area, with the MRE-derived biomarkers showing a higher level of significance (Fig. 2). However, there was no significant correlation between the change in any MRE biomarker and change in tumour ADC.

Discussion:

Clear associations between the MRE biomarkers and ADC values with tumour pathology were apparent from the parametric maps. The significant correlations between the change of hyper-signal area (altered pathology) and the change of viscoelasticity or water diffusivity (perturbed imaging biomarker) confirms that both MRE and DWI are interrogating intrinsic tumour biophysical properties. Although tumour progression and treatment response were associated with MRE changes (decreased $|G^*|$, increased attenuation α), as well as increased ADC, there was no correlation between per-tumour MRE and ADC changes. This could not be explained merely by the intrinsic reproducibility of these respective imaging biomarkers. It is thus reasonable to infer that MRE and ADC are interrogating different underlying biophysical properties of tumours, and together provide complementary information. For this particular pathological phenotype it appears that in the hyper-signal area, cell shrinkage and loss of connectivity to each other or to the extracellular matrix, contributed to alteration of tissue mechanical properties, despite the maintenance of the collagen network, whilst the barriers to water diffusion in this region were less perturbed. The stronger and more significant (higher p value) correlation between the pathological change and $|G^*|$ should make MRE determined $|G^*|$ a potentially more sensitive, and hence earlier, biomarker of tumour response than ADC.

Conclusion:

MRE measured tissue viscoelastic properties and ADC are robust imaging biomarkers of cell death, and may interrogate different biophysical characteristics. $|G^*|$ is a potentially earlier biomarker of cell death than ADC.

References:

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Acknowledgements:

We acknowledge support from a Dorothy Hodgkin Postgraduate Award (DHPA) EP/P505828/1, The Institute of Cancer Research CR-UK and EPSRC Cancer Imaging Centre, in association with the MRC and Department of Health (England) grants C1060/A10334, NHS funding to the NIHR Biomedical Research Centre, and The Wellcome Trust grant #091763Z/10/Z

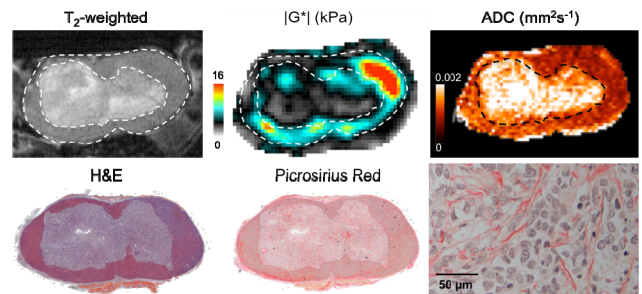


Figure 1. Top: T₂-weighted, $|G^*|$ and ADC images from a BT474 xenograft 24 hours after treatment with AT13148, with the tumour boundary and hyper-signal areas indicated (---). Bottom: whole tumour H&E and picrosirius red stained sections, with an image (×200) of the necrotic area from the picrosirius red stained section.

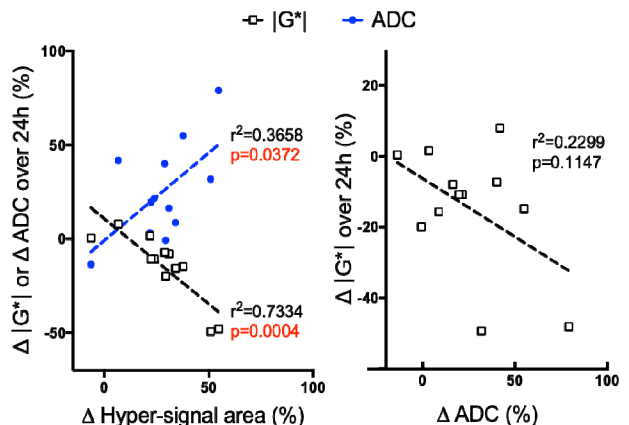


Figure 2. Relationship between tumour changes in $|G^*|$, ADC and percentage hyper-signal area (percentage of the whole tumour) over 24 hours. Correlation between the per-tumour changes of ADC and $|G^*|$.