A novel application of Magnetisation Transfer MRI for the detection of tumour stromal reaction in spontaneous and transplanted Pancreatic Ductal Adenocarcinoma in mice.

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Background and Aims

Pancreatic Ductal Adenocarcimona (PDA) accounts for ~95% of pancreatic cancer cases and is a lethal disease with a median survival period of 6-11 months and a 5-year survival rate of <5%. Treatment with gemcitabine, currently the standard-of-care-drug clinically, is beneficial in only a minority of cases and until recently, the development of more effective treatments has been hindered by a lack of suitable preclinical *in vivo* tumour models. The genetically engineered KPC mouse conditionally expresses endogenous mutant *K*ras and *p*53 alleles under the control of a pancreas-specific *Cre* recombinase. These mice develop spontaneous pancreatic ductal adenocarcinoma (PDA) *in situ* at an average age of 3.5 - 5 months and the tumours recapitulate the genetic, molecular and pathological features of human PDA, notably the low vascular density, high desmoplastic stromal content and poor sensitivity to gemcitabine [1]. Subcutaneous transplantation of cell lines derived from excised KPC tumours gives rise to tumours of the same genotype. However these transplanted PDA tumours have contrasting architecture with minimal desmoplastic stroma, higher vascular density and greater gemcitabine sensitivity. Recently published data show that treatment with gemcitabine in combination with a small molecule hedgehog pathway (Shh) inhibitor depletes the desmoplastic stroma typical of spontaneous PDA and prolongs survival [1]. We hypothesised that the macromolecules present in the tumour stroma would be the prime contributor to the magnetisation transfer. To the authors' knowledge, Magnetisation Transfer (MT-) MRI has not been used to study tumours of the mouse abdomen. The aims of this study were to determine whether MT- MRI, and specifically the Magnetisation Transfer Ratio (MTR), can be used to distinguish between PDA tumours of differing stromal contents and to attempt to use MTR as a translatable imaging biomarker for tumour stromal response to therapy.

Methods

Tumour bearing mice (n=10 per group) were anaesthetised using isoflurane delivered in oxygen. Excess gases were scavenged. An antispasmodic was administered at a dose of 8mg/kg to all mice to prevent gut peristalsis from compromising MR image quality. Mice were scanned in a 40mm Millipede coil in a Varian 9.4T horizontal bore magnet. High resolution T_2W FSE images were acquired from tumour and paraspinal muscle (TR=2000ms, TE_{eff}=25ms, ETL=4, 512x256 points, FOV 80x40mm, slice thickness/gap 1.0/1.0mm, 9 slices) with chemical shift-selective fat suppression and respiratory gating. Magnetisation transfer images were matched to the slice positions and FOV of the anatomical images. For MT-MRI, images were acquired with MT "OFF" (80kHz offset, 400° flip angle) and MT "ON" (4kHz offset, 1350° flip angle) (TR 280ms, TE 2.20ms, 128x128 points, FOV 80x40mm, slice thickness/gap 1.5/0.5mm). MTR shows the fractional change in signal when MT weighting is applied and was calculated by: MTR = (MT "OFF"-MT "ON")/MT "OFF" [2]. MT-MRI analysis was conducted in ImageJ 1.43u (National Institute of Health, USA). ROIs were drawn around tumour and paraspinal muscle tissue on T_2W images, and transferred to the MTR maps. Muscle (n=16) was chosen as a reference tissue based on its inherently high macromolecular content. Mean MTR data were calculated from all pixels in the tumour/muscle regions of interest. Transplanted tumours frequently exhibited cystic and/or necrotic regions clearly visible on T_2W images, which were excluded from the MT analysis; this was rare in spontaneous tumours. The tumours enrolled were >400mm^3 and tumour volumes were calculated by summing the tumour areas in each slice and extrapolating the volume of the gap. In a pilot study one mouse with spontaneous PDA was treated with gemcitabine (100mg/kg) in combination with a small molecule Shh inhibitor (40mg/kg) following the treatment regime previously outlined in [1] and then MR imaged by the protocol described above. Second Harmonic Generation (SHG) microscopy was performed on unstained tissue sections to specifically visualise tissue macromolecules in each untreated tumour group and the single treated spontaneous PDA tumour [3].

Results

MTR distinguishes between tissues of differing macromolecular contents (figure 1). Whole tumour mean MTR data for spontaneous PDA and transplanted PDA were significantly different (p=0.0002). Mean MTR data for muscle taken from mice bearing either spontaneous PDA or transplanted PDA were not significantly different (figure 1). Preliminary data suggest that MTR is sensitive to therapy-induced depletion of the desmoplastic stroma in a single spontaneous PDA tumour (figure 2). Combination therapy did not affect the MTR of paraspinal muscle in the same mouse (figure 2). The *in vivo* MTR data are corroborated by *ex vivo* SHG microscopy which demonstrates the typically dense desmoplastic stroma of spontaneous PDA (figure 3A) compared with that of transplanted PDA (figure 3C). SHG microscopy confirms that combination therapy depleted the stroma of the single treated spontaneous PDA tumour (figure 3B).

Figure 1: Magnetisation Transfer Ratio distinguishes between tissues of different macromolecular contents.

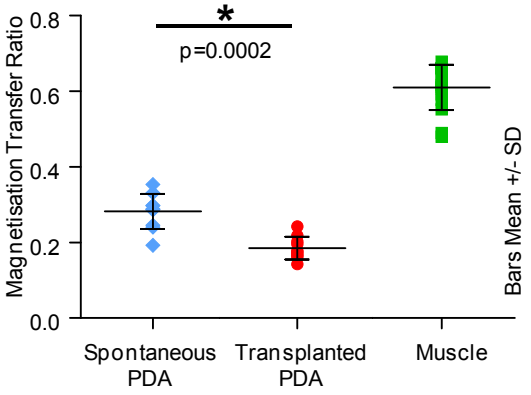


Figure 2: Preliminary data suggest that Magnetisation Transfer Ratio is sensitive to chemotherapy-induced changes in the macromolecular content of spontaneous PDA.

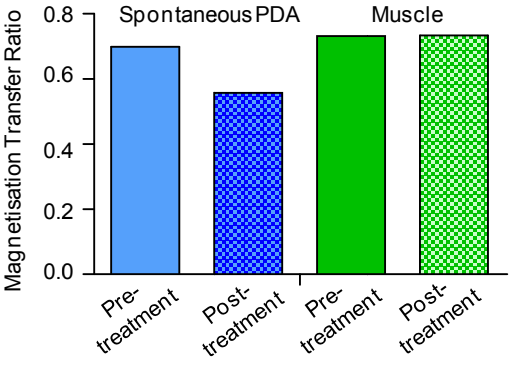
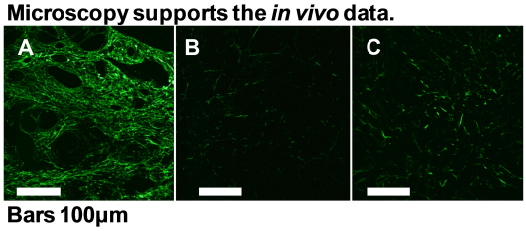


Figure 3: Second Harmonic Generation Microscopy supports the *in vivo* data.



Bars 100µm

Conclusions

MTR is an effective *in vivo* biomarker for the differing macromolecular content in spontaneous and transplanted mouse PDA. Preliminary data suggest that MTR may also be an effective biomarker for detecting tumour stromal depletion in response to treatment. However, further examples of this effect must be obtained to confirm whether or not MTR is a potentially translatable imaging biomarker for the macromolecular content of human PDA in a therapeutic setting. We are currently acquiring multiple MT weightings and implementing quantitative MT analysis to assess tissue macromolecular content more accurately.

References

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