

Acquired resistance to sunitinib is not associated with rescue angiogenesis in 786-O renal cell carcinoma xenografts

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Introduction

Anti-angiogenic therapy has shown considerable efficacy in metastatic renal cell carcinoma (mRCC). Newly diagnosed mRCC patients are now treated with VEGF receptor kinase inhibitors such as sunitinib as standard of care. However, the ability of these drugs to delay tumour progression and extend survival is limited, due to the presence of drug resistance¹. Two modes of resistance to anti-angiogenic therapy are currently recognised, innate resistance, whereby the tumour fails to respond to the therapy from the outset, and acquired resistance, whereby after a period of response to therapy the tumour begins to regrow². Both forms of resistance are thought to arise due to the presence of alternative mechanisms of tumour vascularisation ("rescue angiogenesis"), which are VEGF-independent, and allow the tumour to evade the effects of the targeted agent. However, these mechanisms are poorly understood, and there are currently no validated biomarkers that predict which mRCC patients will benefit from anti-angiogenic therapy. In investigating the response of 786-O RCC xenografts to sunitinib, a model of acquired resistance has been established with chronic treatment. The aims of this study were to i) evaluate fractional blood volume (fBV) derived from susceptibility contrast MRI with ultrasmall superparamagnetic iron oxide (USPIO) particles as a non-invasive imaging biomarker of 786-O xenograft response *in vivo*³, and ii) interrogate the vascular phenotype of 786-O xenografts exhibiting acquired resistance to sunitinib.

Materials & Methods

Female CB17/SCID mice were injected subcutaneously in the flank with 3×10^6 786-O RCC cells. Mice bearing established xenografts ($\sim 150\text{mm}^3$, n=9) underwent MRI prior to and two weeks after daily treatment with 40mg/kg sunitinib p.o. MRI data were acquired on a 7T microimaging system (Bruker Instruments, Ettlingen, Germany) using a 3cm birdcage coil. Following acquisition of contiguous RARE T_2 -weighted MR images for tumour localisation and volume determination, multiple gradient recalled echo (MGRE) images were acquired from a 128x128 matrix over a 3cm FOV, with TR=200ms, TE=6 to 27ms and 8 echoes. MGRE images acquired from three 1mm thick axial slices across each tumour prior to and 3 minutes after intravenous injection of 150 $\mu\text{molFe}/\text{kg}$ of the USPIO particle preparation P904 (Guerbet, Villepinte, France). Parametric R_2^* maps were calculated voxelwise for each tumour slice and fBV (%) determined from an ROI over the whole tumour using in-house software (ImageView)⁴. Functional tumour vasculature was independently quantified by fluorescence microscopy of the uptake of the perfusion marker Hoechst 33342. Additional tumour-bearing mice exhibiting acquired resistance to sunitinib (designated 786-O-R) were similarly imaged when their tumours reached at least 4x their volume at commencement of treatment (n=4).

Results

Calculated fBV maps from one 786-O xenograft prior to and 2 weeks after daily treatment with sunitinib are shown in Figure 1. Susceptibility contrast MRI revealed a reduction in USPIO particle uptake in all treated tumours, resulting in a highly significant (**p<0.01) reduction in fBV in the absence of any change in tumour volume (Table 1). This response was associated with a significant reduction in Hoechst 33342 uptake (*p<0.05). The average pre-treatment tumour fBV was significantly positively correlated ($R^2=0.92$, p<0.0001) with sunitinib-induced changes in tumour fBV across the cohort. Resistant 786-O-R xenografts revealed a suppressed fBV whilst still being treated with sunitinib (Figure 2).

Figure 1

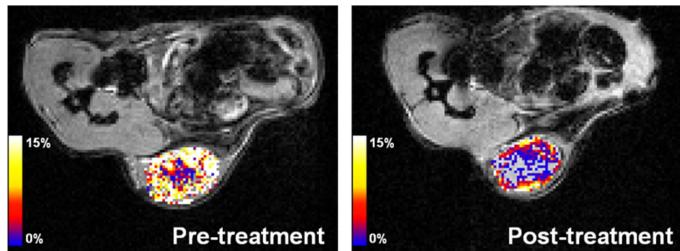
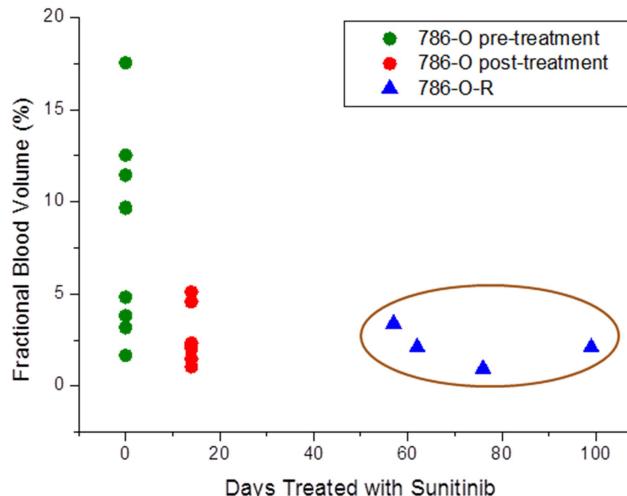


Table 1	786-O		786-O-R
	Pre	Post	Post
Volume (mm^3)	153 ± 17	148 ± 24	471 ± 63
fBV (%)	8.3 ± 2	$2.4 \pm 0.5^{**}$	2.1 ± 0.5
fBV / Volume	6.8 ± 2	$2.5 \pm 1^*$	0.5 ± 0.1
H33342 uptake (%)	13.5 ± 5	$6.4 \pm 1^*$	n.a.

Figure 2



Conclusions

The anti-angiogenic response of 786-O RCC xenografts to sunitinib can be assessed by susceptibility contrast MRI. The sunitinib induced reduction in tumour fBV was associated with reduced Hoechst 33342 uptake, validating fBV as an MRI biomarker of response. Pre-treatment tumour fBV appears to be a predictive biomarker of subsequent response to sunitinib in RCC, whereby more vascular tumours are more susceptible to treatment. Acquired resistance to sunitinib is not associated with tumour re-vascularization in 786-O RCC xenografts.

1) Loges *et al*, *Cancer Cell* 15, 167, 2009; 2) Bergers & Hanahan, *Nat Rev Cancer* 8, 592, 2008; 3) Robinson SP *et al*, *Int J Radiat Oncol Biol Phys* 69, 872, 2007; 4) Walker-Samuel *et al*, *Int J Cancer* 130, 1284, 2011.

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