The Role of Platelet-Derived Growth Factor on Tumour Vascular Function and Morphology *In Vivo* Assessed by Susceptibility Magnetic Resonance Imaging

S. P. Robinson[1], C. Ludwig[2], J. Paulsson[3], J. R. Griffiths[1], A. Ostman[3]

[1]Division of Basic Medical Sciences, St. George's, University of London, London, United Kingdom, [2]Division of Cancer Studies, University of Birmingham, Birmingham, United Kingdom, [3]Department of Pathology-Oncology, Karolinska Institute, Stockholm, Sweden

Introduction

Platelet-derived growth factor (PDGF) receptor signalling contributes to numerous processes in solid tumour progression, including the autocrine stimulation of tumour cell growth, recruitment of fibroblasts and pericytes, and stimulation of tumour angiogenesis[1]. The effects of PDGF expression on tumour vascular function and morphology, in which paracrine expression of PDGF-B by tumour cells results in increased pericyte abundance and enhanced growth rate in the absence of any increased vessel density[2], were investigated by *in vivo* magnetic resonance imaging (MRI). Specifically, intrinsic-susceptibility MRI, sensitive to changes in endogenous deoxyhaemoglobin, was used to investigate the effects of PDGF on tumour vascular maturation and function[3,4]. In addition, preliminary susceptibility-contrast enhanced MRI measurements were made to assess relative tumour blood volume and vessel size index[4-6].

Methods

Tumours derived from B16 melanoma cells transfected with either control plasmid (B16) or plasmid encoding full-length PDGF-B (B16/PDGF) were grown in the flanks of adult C57 Bl6/J mice. The B16/PDGF tumours exhibited a faster growth rate compared to the B16 tumours. The transverse relaxation rate R_2* of size-matched (~750mg) B16/PDGF and B16 tumours was quantified whilst the host first inhaled 5% CO_2/95% air, (for vascular maturation), and subsequently the hyperoxic gas 5% CO_2/95% O_2, (for vascular function)[3]. Tumour blood vessels containing pericytes are expected to either vasodilate with hypercapnia or counteract any hyperoxia-induced vasoconstriction, resulting in a decrease in R_2*. Signal changes in response to hyperoxia are expected in functional blood vessels due to a reduction of paramagnetic deoxyhaemoglobin, causing a further reduction in R_2*. Quantitation of tumour R_2* was performed using a Varian Unity Inova spectrometer interfaced to a 4.7T horizontal magnet, using a 3 turn 15mm solenoid ^{1}H coil. Multi gradient-echo (MGRE) images were acquired from three 1mm thick transverse slices through the tumour, with TR=80ms, TE=3.5ms, TESPACE=3ms and 8 echoes. Apparent R_2* maps were calculated on a voxel-by-voxel basis and R_2* determined from an ROI over the whole tumour.

For susceptibility contrast MRI, the same MGRE sequence and a multi spin echo sequence, with TE=11, 20 and 40ms, were used to determine tumour R_2* and R_2. Diffusion weighted spin echo images were acquired with the diffusion gradient along the three main axes and the apparent diffusion coefficient (ADC) map calculated using the image with the least motion artefact. Tumour R_2* and R_2 maps were acquired prior to and post injection of 2.5mgFe/kg of the USPIO blood pool contrast agent NC100150 via a lateral tail vein. After gaussian smoothing, maps of USPIO-induced ΔR_2* and ΔR_2 were combined with the ADC map to calculate the relative tumour blood volume (rBV, %) and vessel size index (VSI, μm) over a global tumour ROI[5].

Results & Discussion

R_2* maps acquired from a B16/PDGF tumour whilst the host breathed air, 5% CO_2/95% air and 5% CO_2/95% O_2 are shown below, and the intrinsic-susceptibility MRI data summarised in Table 1 (mean ± 1 s.e.m.). Surprisingly, hypercapnia induced a negligible reduction in R_2* of both B16/PDGF and B16 tumours, raising concerns on the utility of intrinsic-susceptibility MRI to assess tumour vascular maturation status. In contrast, hyperoxia resulted in significant reductions in R_2* of both tumour types (*$p<0.03$), and this reduction was significantly greater in the B16/PDGF melanomas ($^{\#}p<0.02$), consistent with increased pericyte coverage through PDGF expression increasing blood vessel function *in vivo*.

The susceptibility contrast data acquired to date are summarised in Table 2 (mean ± 1 s.e.m.). A greater rBV and smaller VSI was found in the B16/PDGF tumours compared to B16 control tumours. These data suggest a PDGF-dependent increase in tumour blood volume and reduction in vessel calibre, consistent with stereological measurements in the same tumour model system[2].

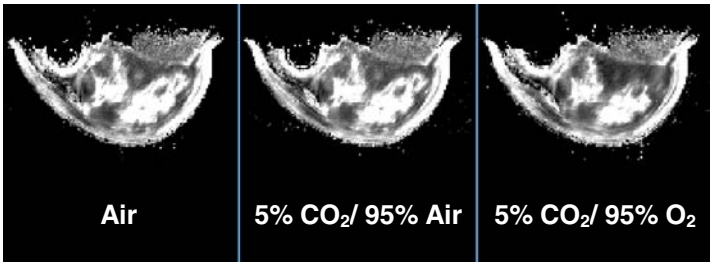

Table 1	**B16 (n=6)**	**B16/PDGF (n=5)**
Baseline R_2* (s^{-1})	70.1 ± 6	63.8 ± 7
ΔR_2* (Air – CO_2) (s^{-1})	-0.9 ± 0.4	-0.1 ± 0.3
ΔR_2* (CO_2 – O_2) (s^{-1})	-3 ± 1*	-7.1 ± 1*$^{\#}$

Table 2	**B16 (n=9)**	**B16/PDGF (n=9)**	**p**
rBV (%)	2.9 ± 0.3	4.2 ± 1	0.06
VSI (μm)	11.5 ± 1	9.02 ± 1	0.07
ADC (10^{-9} m^2 s^{-1})	0.74 ± 0.04	0.94 ± 0.09	0.07

Together these data highlight the role of PDGF in tumour blood vessel function and morphology *in vivo,* and that PDGF represents an attractive target for anti-angiogenic therapies. PDGF is also implicated in the elevated interstitial fluid pressure (IFP) of solid tumours[7]. Treatment with a putative PDGF antagonist has been shown to decrease IFP, and which correlated with a reduction in relative tumour blood volume measured by contrast-enhanced MRI[8]. A hyperoxia-induced ΔR_2*, which also reflects tumour blood volume[6], may also provide a surrogate biomarker for drug-induced change in tumour IFP, a concept currently being persued.

1) Pietras *et al*, Cancer Cell, 3, 439, 2003. 2) Furuhashi *et al*, Cancer Res, 64, 2725, 2004. 3) Abramovitch *et al*, Cancer Res, 59, 5012, 1999. 4) Kostourou *et al*, Cancer Res, 63, 4960, 2003. 5) Ludwig *et al*, Proc. ISMRM, #1230, 2003. 6) Robinson *et al*, J Magn Reson Imag, 17, 445, 2003. 7) Heldin e*t al*, Nature Rev Cancer, 4, 806, 2004. 8) Ferretti *et al*, Proc. AACR-NCI-EORTC Meeting of Mol. Targets & Cancer Ther., 2004.

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