Longitudinal studies of angiogenesis in hormone dependent Shionogi tumours

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Introduction:

Tumour microenvironment is an essential factor in the biology and physiology of solid tumours and contributes to diagnostic features and therapeutic response. Particularly important is the tumour blood supply, as many of the physiological parameters, like tissue oxygenation, metabolism, pH and nutrient supply, directly or indirectly depend on it. By measuring the increase in the transverse relaxation rates ΔR_2 and ΔR_2* caused by the injection of an ultra-small superparamagnetic iron oxide (USPIO) contrast agent, it is possible to measure a weighted average of the blood vessel radii, known as the Vessel Size Index (VSI) [1]. Many solid tumours are hormone dependent. Several studies in animal models of hormone dependent tumours suggested strong correlation between the hormonal status and tumour microenvironment, including blood supply. In this study we evaluated the feasibility of using VSI techniques to measure long term changes in blood supply of subcutaneous tumours and to assess possible correlation between angiogenesis and the tumour's hormonal status in Shionogi model.

Materials and Methods:

Seventeen male DD/S mice had Shionogi tumour cells implanted subcutaneously on the lower back. At least two VSI measurements for each of the stages of tumour progression (growth, regression and relapse) were attempted. Imaging was performed on a 2.35 T, 40 cm bore magnet (Bruker) equipped with a SMIS console using a 2.5 cm diameter 3 turn solenoid coil. Mice were anaesthetized with 1.5 % isoflurane in air. Body temperature was maintained at 35 to 37 ^{o}C using a hot water heating pad. Remote injection of 200 µmol Fe/kg of the contrast agent (ferumoxtran-10, Advanced Magnetics, Cambridge MA) was accomplished using a 27 gauge x 1/2 inch long needle inserted into the tail vein and attached to 0.38 mm inner diameter polyethylene tubing. Imaging was performed in the transverse plane on a 2 mm thick slice through the centre of the tumour using 128 x 128 matrix and a FOV of 40 mm. For ΔR_2 measurements, a spin echo sequence with TR/TE = 2500/60 ms was used and for ΔR_2* measurements, a gradient echo sequence with TR/TE = 2500/10 ms was used. The ADC was measured using a diffusion weighted spin echo sequence with TR/TE = 2500/60 ms and b = 1000 s/mm^2. A multi slice proton density weighted (TR/TE = 2500/10 ms) sequence was used for tumour volume measurements. The tumour VSI was calculated by measuring the total signal from an ROI encompassing the entire tumour in the slice and using that to calculate the ΔR_2, ΔR_2*, and ADC values for the tumour.

Results and Discussion:

The increase in blood susceptibility caused by the contrast agent, $\Delta\chi$, was measured to be 6.37 ± 0.15 ppm (SI). In the lower limit, the static dephasing and slow diffusion approximations limit measurements of VSI to values greater than 7 µm [1]. The upper limit of validity for VSI measurements is based on the need for sufficient averaging over the voxel for the analytical spin echo equation to be valid, thus setting an upper limit of 50 µm based on an ROI encompassing the tumour on a 2mm slice [2]. Only 6 of the tumours successfully progressed to an androgen independent state. Statistical analysis using Spearman rank correlation test showed no dependence between the vessel size and the tumour volume at any stage of the tumour growth cycle (see Figure 1). The average VSI for each stage of tumour progression was calculated using all the measurements made on mice in that stage and is shown in Table 1. These measurements and trend appear to be consistent with results obtained using microscopy [3]. In the 13 mice whose tumours regressed, all but two saw their mean VSI decrease post castration, and of the six mice that progressed to an androgen independent state, all but one experienced a subsequent increase in tumour VSI (see Figure 2). The longitudinal nature of this study lends itself well to an analysis that takes into consideration the dependant nature of the measurements; hence a paired Student's t test was used to test the significance of changes in the tumour VSI. With a t-statistic of 3.205 (P = 0.0076) there is strong evidence to support a decrease in vessel size post castration in the regressing tumour. The t-statistics for a difference between regressing and relapsing and growing and relapsing tumours are –2.417 (P = 0.067) and –0.215 (P = 0.838) respectively.

	VSI (µm)	σ	n
Growing	35.2	25.5	30
Regressing	15.1	6.56	22
Relapsing	45.4	41.8	11

Table 1: Average tumour VSI measurements in each of the stages of Shionogi tumour progression.

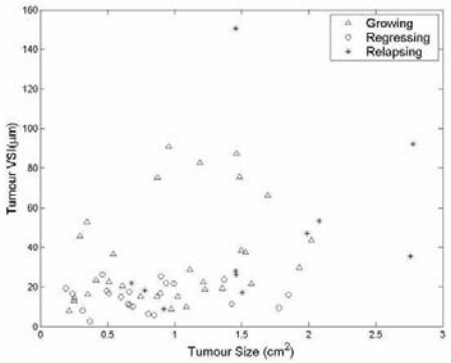

Figure 1: Vessel Size Index appears to be independent of tumour size. Overall Spearman rank correlation coefficient is $r_s = 0.386$ (P = 0.003).

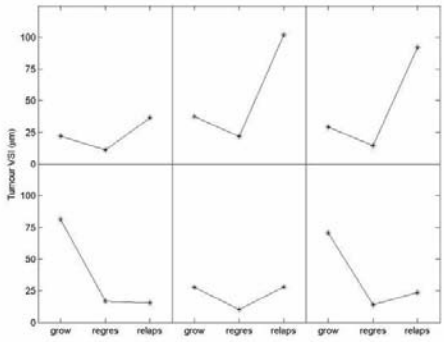

Figure 2: Tumour VSI trends of the 6 mice that progressed to an androgen independent state.

Conclusion:

Vessel size imaging has proved to be a viable method of monitoring angiogenesis during the progression of a Shionogi tumour from androgen dependence to androgen independence. It appears to work well for subcutaneous tumours and proven capable of distinguishing the differences in vasculature between the different stages of tumour progression

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