VEGF Overexpression Alters Co-Localization Patterns Of Vascular And Metabolic Parameters

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SYNOPSIS: Vascular endothelial growth factor (VEGF-A) is a potent angiogenic and permeability factor and its expression has been linked to metastasis in clinical studies. Here for the first time we have investigated the effect of VEGF overexpression on the relationship between vascular and metabolic parameters in a human prostate cancer model, using combined MRI and MRSI. VEGF overexpressing tumors exhibited patterns of co-localized vascular volume, permeability, total choline and lipid which were distinct from control tumors. These data suggest that VEGF overexpression can influence the choline and lipid metabolism of tumors.

INTRODUCTION: The vascular, physiological and metabolic environment can influence invasion and metastasis. What is not known are the dynamics between these parameters, and if there are combinations of these parameters which represent the most 'permissive' regions for invasion and metastasis. In separate studies we have detected significant differences in vascular, physiological and metabolic characteristics of metastatic and non-metastatic human breast and prostate cancer models with MRI and MRS. Since increased expression of VEGF has been linked to increased metastases [1], in this study we have examined differences in the combinations of vascular and metabolic/physiological regions in tumors overexpressing VEGF. Such combinations, if identified, may represent regions of high 'metastatic threat'.

METHODS: The full-length cDNA for VEGF-A (pHUVEGF.21) was obtained from Genentech. Stably transfected human prostate cancer PC-3 cells containing the VEGF-A gene under a CMV promoter were derived. Combined MRI studies were performed on PC-3 tumors overexpressing VEGF-A (n=3) and on vector transfected control tumors (n=3). ELISA assays were performed to determine VEGF levels in cells and solid tumors (n=6 per group). Co-registered maps of vascular volume, permeability, total choline and lipid were obtained from the tumors. Imaging studies were performed on a Bruker Biospec 4.7T system. Spectroscopic images were acquired from a 2mm thick slice with an in-plane spatial resolution of 1mm×1mm (zero-filled once). Spectral parameters were TE=136ms, TR=1s, NA=4 per phase encode step, FOV=32mm, 16×16 matrix. Water suppression was achieved using VAPOR. Following MRSI, co-localized multi-slice MR T1 relaxation rates of the tumor were obtained by a saturation recovery method combined with fast-T1 SNAPSHOT FLASH, using a solenoid coil placed around the tumor. T1 maps were obtained from four, 1mm slices, starting at 3min post-injection, up to 34min. Relaxation maps were reconstructed from data sets for three different relaxation times and the M_o data set on a voxel-wise basis. At the end of the imaging, animals were sacrificed, 0.5ml of blood withdrawn from the inferior vena cava, and tumors and lungs excised, and fixed in 10% buffered formalin for histology. Vascular volume and permeability surface area product (PSP) maps were generated from the ratio of $\Delta(1/T1)$ values in the images to that of blood. The slope of $\Delta(1/T1)$ ratios versus time was used to compute PSP while the y-intercept was used to compute vascular volume on a voxel-wise basis. Parametric maps of vascular volume, permeability and total choline or lactate/lipid obtained from the voxels of VEGF overexpressing and control tumors were used to generate three dimensional volumetric histogram matrices. Control and overexpressing tumor data are displayed in red and green, respectively. This display extends the conventional histogram plot to a volumetric histogram. Each voxel in the 3D display corresponds to an entry in a three dimensional matrix of vascular volume, permeability and total choline or lactate/lipid. The x-axis of the display represents vascular volume, the y-axis represents permeability, and the z-axis represents total choline or lactate/lipid. The matrix is created by dividing the range of vascular volume, permeability and total choline or lactate/lipid data into 32 bins each, to form a 32×32×32 3D-matrix. The matrix is populated by adding the count to each division from the spatially aligned 3D volume image data sets of vascular volume, permeability and total choline or lactate/lipid. Proton density images (M_o maps) corresponding to each volume data are used to exclude regions outside of the tumor. The color intensity of voxels in the display represents the frequency of occurrence (histogram count) of the entry from the sampled data set.

RESULTS: VEGF levels were two fold higher in the overexpressing tumors compared to control tumros (2.8±0.54 vs 1.2±0.34 pg/ml/µg protein, n=6, $p<0.03$). Similarly, values of vascular volume and PSP were significantly higher ($p<0.05$) in the overexpressing tumors compared to control tumors (9.8±1.7 µl/gm vs 6.2±1.1 ul/gm and 0.23±0.05 vs 0.086±0.025 µl/gm.min, n=5 overexpressing and 4 control tumors).

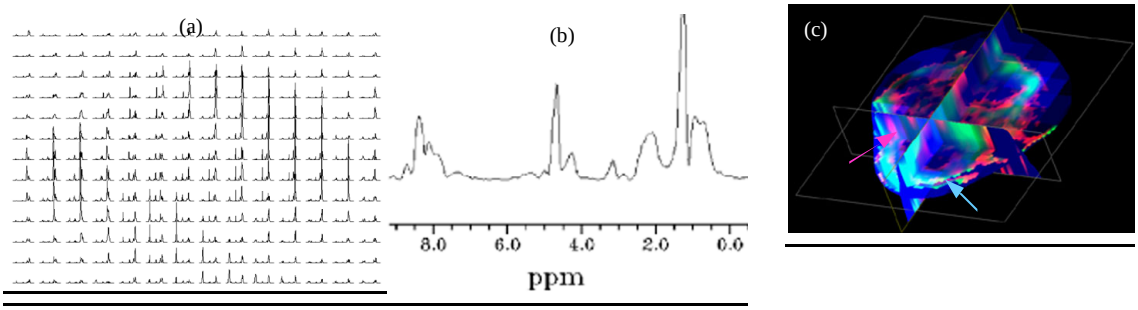

Figure 1: (a) CSI data set from a 2mm thick slice obtained from a VEGF overexpressing PC-3 tumor (volume 273mm^3) with an in-plane resolution of 1mm×1mm (zero-filled once). (b) Spectrum from a 1mm×1mm×2mm voxel. (c) Triplanar view of 3D reconstructed maps of co-localized vascular volume in red, permeability in green and lactate/lipid in blue.

An example of a CSI data set from a VEGF overexpressing tumor is shown in Figure 1a and a representative single voxel is shown in Figure 1b. Total choline at 3.2ppm and lactate/lipid at 1.3 ppm are evident in the Figure 1b. A triplanar view of a 3D reconstructed map of co-localized vascular volume in red, permeability in green and lactate/lipid in blue is shown in Figure 1c.

Regions of cyan approaching white indicating high permeability, high lactate/lipid and relatively high vascular volume, as well as magenta (high vascular volume and high lactate/lipid) are evident in the figure. Significant differences were detected in the 3D volumetric histograms generated from co-localized vascular and metabolic maps (Figure 2). There were voxels in the overexpressing tumors with high lipid and high permeability as well as high choline and high permeability.

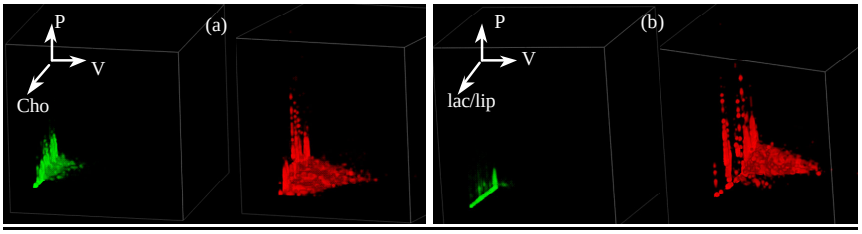

Figure 2: 3D volumetric histograms of (a) vascular volume, permeability and total choline in control (green) and VEGF (red) tumors, and (b) vascular volume, permeability and lactate/lipid in control (green) and VEGF (red) tumors.

DISCUSSION/CONCLUSIONS: Consistent with our previous results, VEGF overexpression significantly increased vascular volume and permeability. VEGF overexpression also significantly increased permeability of regions with high vascular volume, which are not very leaky in wild type and control tumors. Here for the first time we have demonstrated that VEGF overexpressing tumors exhibited a pattern of co-localized values of vascular volume, permeability, total choline and lipid distinct from control tumors. We are currently quantifying choline metabolites in control and VEGF overexpressing tumors. These data suggest that VEGF expression can influence choline and lipid metabolism in solid tumors.

References: 1. Salven P, et al, *Clin Cancer Res* (3):647-651,1997 **Acknowledgements:** This work is supported by NIH 2 RO1 CA73850. We thank Genentech, CA, for the full-length VEGF cDNA, Dr. Dikoma Shungu for CSI data analyses software, and Gary Cromwell, Yelena Mironchik, and Flonne Wildes for technical assistance.