Differences in Lymphatic Drain Following VEGF Overexpression in a Human Breast Cancer Model

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SYNOPSIS: **Several studies have investigated the effects of VEGF-C and –D on lymphangiogenesis, but few have focused on effects of full-length VEGF-A on the lymphatic continuum. This is the first *in vivo* functional MRI study to noninvasively characterize differences in lymphatic/convective drainage between MCF-7 breast cancer xenografts overexpressing full-length VEGF, and vector transfected control tumors. We detected significantly enhanced lymphatico-convective drainage at tumor-tissue interfaces for VEGF overexpressing MCF-7 tumors compared to control MCF-7 tumors. Consistent with these observations, fluorescence microscopy of the novel lymphatic endothelium marker (LYVE-1) detected a trend of dilated lymphatic vessels in peritumoral regions of VEGF overexpressing tumors.**

INTRODUCTION: Although the role of VEGF-A as a potent vascular permeability factor is well established, a recent study reported that VEGF164-induced existing lymphatics to swell due to interstitial edema caused by elevated vascular permeability [1]. This distension was followed by lymphatic endothelial cell proliferation and formation of sinusoidal lymphatics, suggesting that lymphangiogenesis is induced in response to elevated interstitial pressure arising from edema. Similarly, in a rat-tail model, it was shown that interstitial fluid channeling precedes, and may even direct, lymphangiogenesis [2]. In other studies using cancer models overexpressing VEGF165, significantly increased extravasation of the macromolecular contrast agent albumin-GdDTPA was observed, with drainage towards the nearest lymph node [3]. In addition, clinical data have demonstrated that a link between VEGF-A and nodal metastasis [4], again suggesting a link between VEGF-A and the lymphatic system. Most studies investigating the effects of VEGF are performed with cell lines overexpressing a single isoform e.g. VEGF165. However, to understand the role of VEGF it is critical to use cells overexpressing the full form of VEGF. In this study, we investigated the effect of full-length VEGF-A overexpression on lymphatic or convective drainage using MRI.

METHODS: Full-length cDNA for VEGF-A (pHUVEGF.21) was obtained from Genentech, and stably transfected human breast cancer MCF-7 cells overexpressing VEGF-A were derived. MRI was performed on MCF-7 tumors overexpressing VEGF-A (n=4) and on vector-transfected control tumors (n=5). ELISA assays were performed to determine VEGF levels in solid tumors. Multi-slice MR T1 relaxation rates of the tumor were obtained by saturation recovery method combined with fast-T1 SNAPSHOT FLASH, which provided images of the entire mouse cross-section including the tumor. Five to eight, 1mm slices were acquired with a 256×256μm^2 resolution for three relaxation delays (100, 500 and 1000ms). Images were obtained before i.v. administration of 0.2ml of 60mg/ml albumin Gd-DTPA and repeated every 7 min, starting at 3 min post-injection, up to 30 min, followed by an interval of one hour, after which a second block of data was acquired up to 150 min. Image time courses corresponding to the 1s relaxation delay were analyzed using cross-correlation (*cc*) analyses to identify convective/lymphatic voxels [5,6]. For each animal, we defined a 750μm swathe of tissue at the tumor-normal tissue interface in each post-contrast image slice as our region of interest (ROI). We then determined the total number of lymphatico-convective or drainage voxels in each ROI and expressed it as a fraction of the total number of voxels in the ROI over the entire tumor. For fluorescence microscopy, we used LYVE-1 to identify lymphatic endothelia.

RESULTS: VEGF levels assayed in solid tumors were significantly (p=0.002) greater (12.47±5.2 ng/ml/mg protein) for the VEGF overexpressing clone than the vector transfected clone (0.15±0.1 ng/ml/mg protein). **Fig. 1A** shows a montage of MR images overlaid with cross-correlation (*cc*) maps, shown in yellow (*cc*>0.8) from an MCF-7 VEGF overexpressing tumor, and **Fig. 1B** a MCF-7 control tumor, illustrating differences in the spatial distribution of convective/lymphatic voxels. **Fig 1C** is a cutaway of a 3D reconstruction of a VEGF overexpressing tumor and its *cc* overlay; the clearance of contrast at the tumor-tissue interface is clearly evident (→). There were a significantly (p=0.006) greater number of voxels at the tumor-tissue interface in the VEGF overexpressing MCF-7 tumors than in the control MCF-7 tumors (**Fig. 2A**) as determined by the Mann-Whitney-U test. Fluorescence microscopy of control MCF-7 tumor sections stained with lymphatic endothelium-specific receptor LYVE-1, showed single, tenuous peritumoral lymphatics (**Fig. 2B**), while the VEGF overexpressing tumors exhibited dilated peritumoral lymphatics (**Fig. 2C**). These morphological differences in peritumoral lymphatics between VEGF overexpressing and control MCF-7 tumors are consistent with the differences in the convective/lymphatic drainage at the tumor-tissue interface identified by MRI.

DISCUSSION/CONCLUSIONS: These data suggest that in keeping with its known role as a permeability enhancer, full-length VEGF-A increases extravasation of the macromolecular contrast agent into the tumor interstitium. This leads to an elevation in interstitial pressure and an increase in convection and potential drainage via lymphatic channels at the tumor-tissue interface. The identification of enhanced drainage, via both MRI and fluorescent microscopy for the VEGF overexpressing tumors has the potential to increase our understanding of the mechanistic aspects of tumor metastasis. Currently, we are in the process of characterizing differences in lymph node metastasis for the VEGF overexpressing and control tumors.

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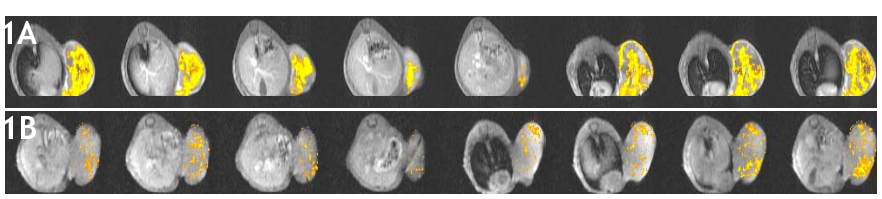

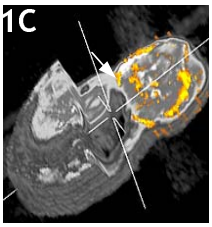

Degree of Drainage at Tumor-Tissue Interface