Differential Effects of VEGF Overexpression on Angiogenesis and ECM Integrity in Breast Cancer Xenografts Pre-selected for Their Invasiveness

A. P. Pathak[1], S. McNutt[1], F. Wildes[1], V. Raman[1], and Z. M. Bhujwalla[1]

[1]JHU ICMIC Program, Russell H. Morgan Dept. of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

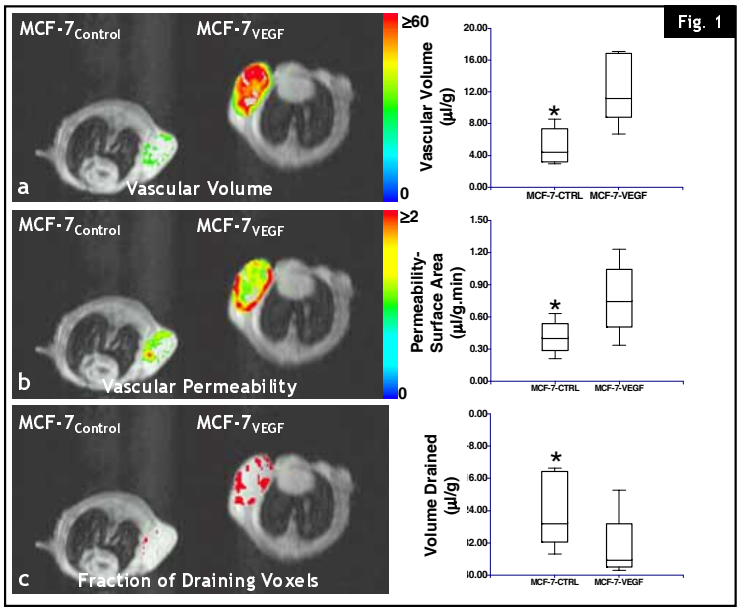
INTRODUCTION: The success of anti-VEGF therapy in patients with metastatic breast cancer, underscores the important role of VEGF in breast cancer [1]. While most studies employ histology to explore the relationship between angiogenesis and VEGF expression in breast cancer [2], few have investigated these effects *in vivo*, and fewer still have assessed the relationship between VEGF expression, angiogenesis and extracellular matrix (ECM) integrity within the context of metastatic breast cancer [3]. We recently demonstrated the feasibility of assessing angiogenesis and ECM integrity *in vivo* using contrast-enhanced MRI [4]. Here, we exploit this approach to characterize angiogenesis (vascular volume/permeability), and ECM integrity (draining/pooling rates) of noninvasive MCF-7 and invasive MDA-MB-231 human breast cancer xenografts engineered to overexpress human vascular endothelial growth factor (VEGF). Our MRI and histological data demonstrate that VEGF overexpression transforms both, noninvasive MCF-7 and invasive MDA-MB-321tumors to a more angiogenic phenotype, but significantly alters ECM integrity only in MCF-7 tumors. These data have significant implications for understanding the correlation between angiogenesis and ECM integrity in metastasis.

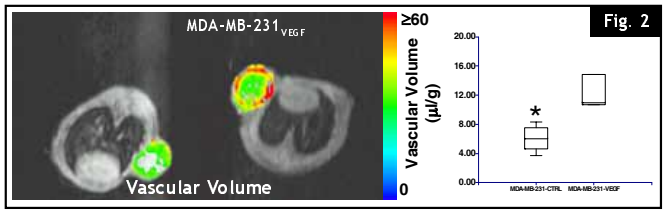
METHODS: Full-length cDNA for human VEGF-A was obtained from Genentech, and stably transfected human breast cancer MCF-7 and MDA-MB-231 cells containing the VEGF-A gene under the control of a CMV promoter were derived. MRI was performed on animals bearing MCF-7 VEGF overexpressing (n=12) and control tumors (n=10) to determine angiogenic parameters, with five animals from each group undergoing the extended imaging protocol to determine ECM integrity. Animals bearing MDA-MB-231 VEGF overexpressing (n=4), and MDA-MB-231 control tumors (n=5) were imaged using the extended imaging protocol. ELISAs were performed to determine VEGF levels in cells/tumors. Multi-slice T_1-relaxation rates of the tumor were obtained by a saturation recovery method combined with SNAPSHOT FLASH. At least five, 1mm slices were acquired ($256\times256\mu m^2$) for relaxation delays of 100, 500, 1000 and 7000ms. Images were obtained before i.v. administration of 0.2ml albumin Gd-DTPA and repeated every 5 min, starting at 3 min post-injection, up to 35 min (or up to 120 min for the extended protocol [4]). After imaging, animals were sacrificed, 0.5 ml of blood withdrawn from the inferior vena cava, tumors excised, and fixed for immunofluorescent microscopy with vascular and lymphatic endothelial cell markers [4]. Maps of vascular volume (VV), permeability-surface area product (PSP), draining and pooling rates, and fraction of draining pooling voxels in the ECM were generated as described in [4]. Stereological analysis of blood/lymphatic vessels was conducted on histological sections.

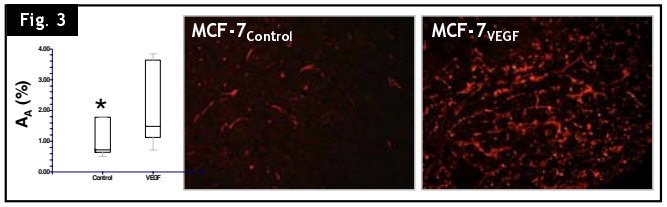
RESULTS: VEGF levels assayed in cells and solid tumors were significantly greater for the VEGF overexpressing clones than vector transfected clones (**Table 1**). MCF-7_{VEGF} tumors showed significantly (two-tailed MW-U p-values of 0.0003, 0.0037 and 0.0047, respectively) higher median VV, PSP and volume of fluid exudate drained, computed over the entire tumor, compared to MCF-$7_{Control}$ tumors (**Fig. 1a-c**). MDA-MB-231_{VEGF} tumors showed significantly (two-tailed MW-U p-value of 0.0014) higher median VV compared to MDA-MB-$231_{Control}$ tumors, but no significant differences in PS or draining rates (**Fig. 2**). Finally, the fractional blood vessel area as assessed by CD34 staining was significantly (two-tailed MW-U p-value of 0.045 and 0.007, respectively) greater for both MCF-7_{VEGF} and MDA-MB-231_{VEGF} tumors, compared to MCF-$7_{Control}$ and MDA-MB-$231_{Control}$ tumors, respectively (**Fig. 3**), but there were no significant differences in fractional intratumoral lymphatic vessel area for either.

Table 1	ELISA on Cells (VEGF in pg/ml/10^6 cells)	ELISA on Tumors (VEGF in pg /μg protein)
Vector Transfected MCF-7	65.5 ± 31	0.153 ± 0.035 (n=6)
VEGF transfected MCF-7	987.8 ± 163	12.47 ± 2.13 (n=6)
Vector Transfected MDA-MB-231	771.5 ± 242	1.722 ± 0.88 (n=3)
VEGF transfected MDA-MB-231	1616.4 ± 1.7	4.6 ± 1.6 (n=3)

DISCUSSION/CONCLUSIONS: The most significant effect of VEGF overexpression was the transformation of MCF-7 tumors to a more angiogenic phenotype, i.e. MCF-7_{VEGF} tumors exhibited elevated vascular volume and permeability compared to MCF-$7_{Control}$ tumors. This is consistent with the role of VEGF as a potent permeability factor. More surprising was that MCF-7_{VEGF} tumors not only exhibited a larger number of voxels from which fluid exudate was being drained in the ECM, but the volume of fluid drained in these tumors was also greater than that of MCF-$7_{Control}$ tumors. These observations are consistent with reports of the extracellular regulation of VEGF by matrix metalloproteinases (MMPs) that accompany ECM remodeling [5] and initiate the "angiogenic switch" [6]. In contrast, although MDA-MB-231_{VEGF} tumors exhibited elevated blood volume compared to MDA-MB-$231_{Control}$ tumors, there were no measurable differences in ECM integrity between the two, consistent with their inherently high baseline MMPs activity levels. We are currently evaluating differences in lymph node and lung metastases between VEGF overexpressing and control tumors to determine if the MCF-7_{VEGF} tumors, with their newly acquired "angiogenic phenotype" in conjunction with their "remodeled ECM" exhibit a *de novo* threat of metastatic dissemination.







REFERERENCES: **1.** Senior K, *Lancet Oncol*, May;7(5):370, 2006. **2.** Weidner N et al., *N Engl J Med*, Jan 3;324(1):1-8, 1991. **3.** Miralem T et al., *Oncogene*, Sep 6;20(39):5511-24, 2001. **4.** Pathak et al., *Cancer Res*, May 15;66(10):5151-8, 2006. **5.** Lee S et al., *J Cell Biol*, May 23;169(4):681-91, 2005. **6.** Bergers G et al., *Nature Cell Biology*, 2:737-744, 2000.