Under Hypoxia, VEGF Overexpression Increased Invasion and Changed Cellular Metabolism of a Human Prostate Cancer Cell Line Co-Cultured with Endothelial Cells

E. Ackerstaff[1], F. Wildes[1], V. Raman[1], D. Artemov[1], Z. M. Bhujwalla[1]

[1]Radiology - MR Research, Johns Hopkins University School of Medicine, Baltimore, MD, United States

Introduction: The potent permeability factor, **v**ascular endothelial growth factor (VEGF), is frequently upregulated in cancer cells [1]. *In vivo*, increased expression of VEGF has been linked to increased occurrence of metastases [2]. VEGF production is stimulated under hypoxia through the binding of HIF-1 to the hypoxia response element in the promoter region of VEGF[1]. We recently generated stably transfected PC-3 cells overexpressing $VEGF_{165}$. Using the VEGF-overexpressing and parental PC-3 cells in our Metabolic Boyden Chamber Assay [3], we investigated the effects of VEGF overexpression on the invasion and metabolism of these cancer cells co-cultured with human umblical vein endothelial cells (HUVECs) under well-oxygenated and hypoxic conditions.

Material and Methods: Parental PC-3 cells, obtained from ATCC, were originally derived from a bone metastasis of a prostate cancer patient. PC-3VEGFc2 cells, i.e. PC-3 cells overexpressing $VEGF_{165}$, were generated using the expression vector pCR3.1 containing human pHuVEGF-21 (Genentech, CA, USA); empty-vector-transfected PC-3 cells (PC-3pCR3.1) served as control. In all NMR experiments, the ECM gel chamber consisted of a layer of polymerized ECM gel with $5x10^4$ HUVECs (Clonetics) on the surface of the ECM gel forming a lumen-like structure. Adherently grown cancer cells were layered on either side of the ECM gel chamber in a customized 10-mm NMR tube and perfused with RPMI 1640 supplemented with 9% fetal bovine serum, 90 U/ml Penicillin, 90 µg/ml Streptomycin, w/ or w/o G418, and 10 mM HEPES. The sample temperature was kept at 37°C. The oxygen tensions were kept above 20 % for oxygenated conditions, and below 1.5 % for hypoxia. Cancer cell invasion was quantified from changes in the profiles of intracellular water along the sample. Cell metabolism was studied along the sample in 310 µm thick slices by 1D ^{1}H spectroscopic imaging, and over the entire sample by 1D ^{1}H MRS and 1D ^{31}P MRS.

Results:

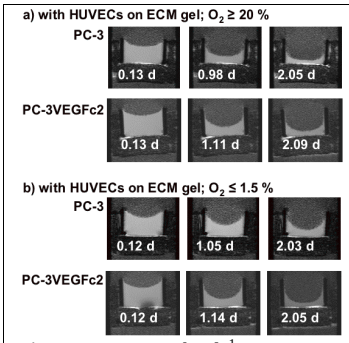

Figure 1: T_1-weighted ^{1}H MR images of PC-3 and PC-3VEGFc2 cells, both combined with HUVECs on the ECM gel demonstrating
(a) similar rates of degradation of ECM gel under oxygenation,
(b) increased degradation of ECM gel under hypoxia by PC-3VEGFc2 cells compared to PC-3 cells.

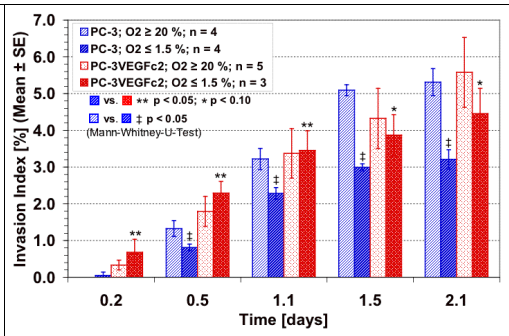

Figure 2: Invasion Index of VEGF-overexpressing PC-3VEGFc2 cells compared to PC-3 cells, both with HUVECs present on ECM gel under well-oxygenated ($O_2 \geq 20$ %) and ($O_2 \leq 1.5$ %) hypoxic conditions.

Table 1: Summarized results obtained from global 1D ^{31}P MR spectra for PC-3VEGFc2 and PC-3 cells under oxygenation or hypoxia.

	With HUVECs on ECM gel			
	$O_2 \geq 20$ %		$O_2 \leq 1.5$ %	
	PC-3	PC-3VEGFc2	PC-3	PC-3VEGFc2
pH	7.25 - 7.30	7.25 - 7.20	7.25 - 7.20	7.25 - 7.20
Pi	↘ to ~ 75 %	↘ to ~ 60 %	↘ to ~ 75 %	↘ to ~ 75 %
PCr	↘ to ~ 85 %	not detectable	↗ x 4	↗ x 4
ß-NTP	constant	↘ to ~ 85 %	constant	constant
PC	↑ x 2.0	↑ x 2.0	↑ x 1.7	↗ x 2.3
GPC	↘	↘	↘	↘

Signal assignments: Pi, inorganic phosphate; PCr, phosphocrea-tine; ß-NTP, ß-nucleoside triphosphate; PC, phosphocholine; GPC, glycerophosphocholine

PC-3pCR3.1 and PC-3VEGFc2 exhibited the same invasiveness under control conditions as parental PC-3 cells (data not shown). As presented in Fig. 1 and Fig. 2, VEGF overexpression did not alter the invasiveness of well-oxygenated PC-3 cells. Under hypoxia, however, VEGF overexpression significantly increased the invasion of PC-3 cells leading to invasion comparable to that of well-oxygenated cells (Fig.2). VEGF overexpression did not change the energy status or intracellular pH of PC-3 cells (Table 1). Hypoxia increased PCr levels in PC-3 cells as well as in PC-3VEGFc2 cells (Table 1).

VEGF overexpression decreased significantly intracellular LacTG levels (Fig. 3). This decrease was primarily due to lowered intracellular levels of triglycerides and not intracellular Lac levels (Fig. 4).

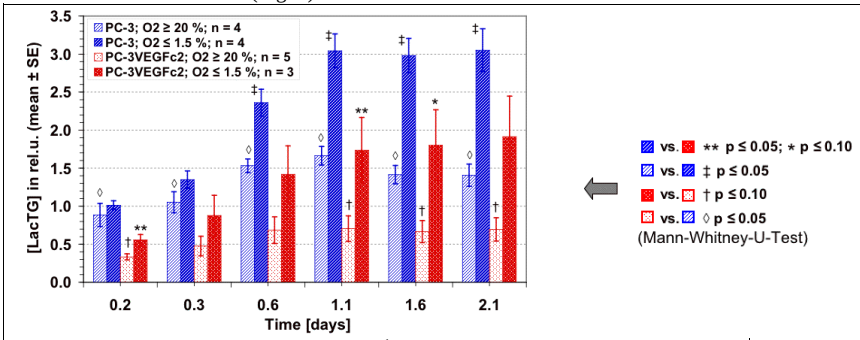

Figure 3: Data obtained from global 1D ^{1}H MR spectra demonstrated that in the presence of HUVECs on ECM gel, intracellular LacTG (Lactate + Triglycerides) levels of PC3VEGFc2 cells were significantly decreased compared to parental PC-3 cells. Hypoxia increased intracellular LacTG levels in the cancer cells compared to well-oxygenated cells.

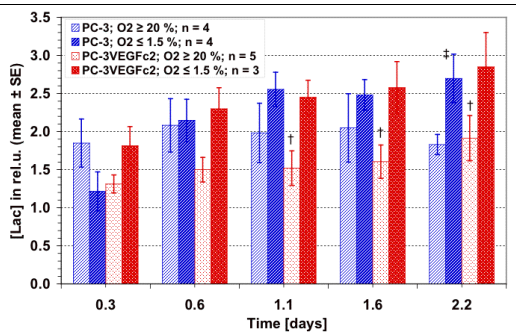

Figure 4: Intracellular Lac levels obtained from global 1D ^{1}H NMR spectra using lactate editing for either PC-3 or PC3VEGFc2 cells co-cultured with HUVECs on ECM gel. Hypoxia increased Lac levels only marginally. No differences in Lac levels were observed as a result of VEGF overexpression.

Discussion: Our results suggest that VEGF overexpression of cancer cells in the presence of endothelial cells may lead to increased cancer cell invasion in regions of the tumor characterized by hypoxia and thus may explain, in part, the increased rates of metastases observed in patients with cancers secreting high levels of VEGF. Independent of VEGF expression levels, intracellular PCr increased under hypoxia, while ATP levels remained constant. These results suggest a shutdown of energy-consuming processes such as proliferation in favor of maintaining ATP levels [5].

References: [1] G.U. Dachs, G.M. Tozer; Eur J Cancer 36:1649-60, 2000; [2] L.M. Ellis, I.J. Fidler; Eur J Cancer 32A(14):2451-60, 1996; [3] U. Pilatus, E. Ackerstaff, D. Artemov, N. Mori, R.J. Gillies, Z.M. Bhujwalla; Neoplasia 2(3):273-79, 2000; [4] E. Ackerstaff, D. Artemov, Z.M. Bhujwalla; ISMRM 2003, Poster #1285; [5] R.G. Boutillier; J Exp Biol 204:3171-81, 2001

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