VEGF Overexpression Increases Invasion of a Human Prostate Cancer Cell Line Under Hypoxia in the Presence of Endothelial Cells.

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Introduction: Vascular endothelial growth factor (VEGF) is a potent permeability factor which is frequently upregulated in cancer cells [1]. Increased expression of VEGF has been linked to increased metastases [2]. VEGF production is also increased under hypoxic conditons through the binding of HIF-1 to a hypoxia response element in the promoter region of VEGF[1]. We recently generated stably transfected PC-3 cells overexpressing full-length VEGF using a cDNA construct (human pHu VEGF.21) obtained from Genentech. In this study we have determined the effects of VEGF overexpression on the invasion of PC-3 cells in contact with human umblical vein endothelial cells (HUVECs) under conditions of normoxia and hypoxia. These studies were performed with our Metabolic Boyden Chamber Invasion Assay [3].

Material and Methods: PC-3 cells were obtained from ATCC. PC-3 cells overexpressing full-length VEGF (PC-3VEGFc2 cells) were generated using the expression vector pCR3.1 containing human pHuVEGF-21 (Genentech, CA, USA) under the control of the CMV promoter; empty-vector transfected PC-3 cells (PC-3pCR3.1) were used as control. Prior to the NMR experiments, $5x10^4$ HUVECs (Clonetics, USA) were seeded on the surface of ECM gel. In NMR experiments, adherently grown cancer cells were layered on either side of a chamber containing ECM gel and HUVECs in a customized NMR tube. The sample was 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 at pH 7.2±0.1 and 37°C. Oxygen tensions in the sample were kept at a minimum of 20 % for oxygenated cells, and below 1.5 % for hypoxic conditions. Cancer cell invasion was quantified from changes in the profiles of intracellular water along the sample. Cell metabolism was studied over the entire sample by 1D ^{1}H MRS and 1D ^{31}P MRS, and in 310 µm thick slices along the sample by 1D ^{1}H spectroscopic imaging.

Results:

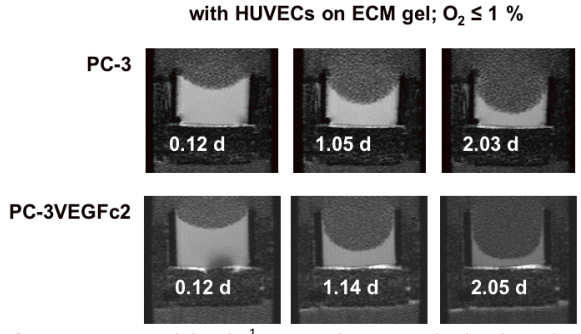

Figure 1: T_1-weighted ^{1}H MR images obtained under continuous hypoxia, with HUVECs on the ECM gel, demonstrating increased degradation of ECM gel by PC-3VEGFc2 cells compared to the parental cell line PC-3.

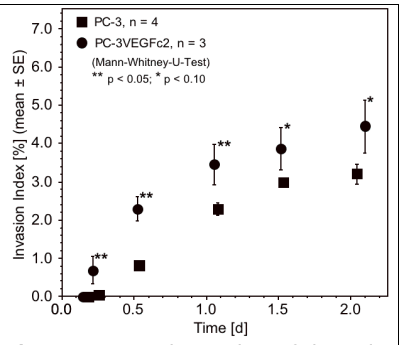

Figure 2: Invasion Index of hypoxic VEGF-overexpressing PC-3VEGFc2 cells compared to hypoxic PC-3 cells, both with HUVECs present on ECM gel.

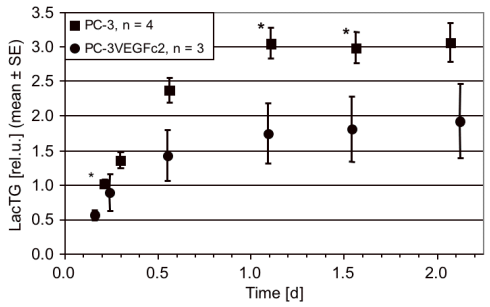

Figure 3: Hypoxia combined with HUVECs on ECM gel significantly decreased intracellular LacTG (Lactate + Triglycerides) levels for PC3VEGFc2 cells compared to PC-3 cells.
($p < 0.05$, Mann-Whitney U Test)

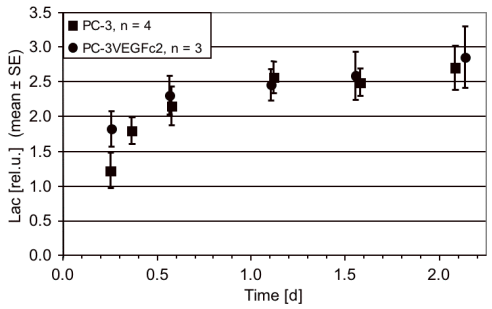

Figure 4: Intracellular Lac levels obtained from global 1D ^{1}H NMR spectra using lactate editing for either PC-3 or PC3VEGFc2 cells in contact with HUVECs on ECM gel under hypoxia. No differences in Lac levels were observed.

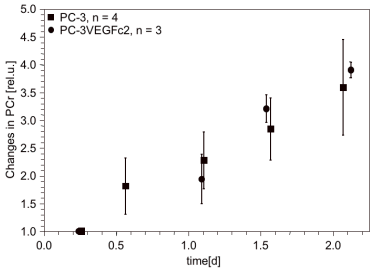

Figure 5: Changes in PCr levels of PC-3 cells and PC-3VEGFc2 cells combined with HUVECs on ECM gel, under hypoxia.

Both PC-3pCR3.1 and PC-3VEGFc2 clones exhibited similar invasiveness under control conditions as wild-type PC-3 cells (data not shown). The presence of HUVECs on ECM gel did not alter the invasion of oxygenated PC-3VEGFc2 cells compared to oxygenated PC-3 cells (data not shown). Under hypoxia, however, the invasion of PC-3VEGFc2 cells compared to wild-type PC-3 cells increased significantly with HUVECs present on ECM gel (**Fig. 1**, and **Fig. 2**).

Under hypoxia, LacTG levels (obtained from global 1D 1H NMR spectra) were significantly lower for PC-3VEGFc2 cells in contact with HUVECs on ECM gel compared to PC-3 cells combined with HUVECs on ECM gel (**Fig. 3**). This decrease was primarily due to decreased intracellular triglycerides levels and not lactate (Lac) levels (**Fig. 4**). Consistent with previous observations made with PC-3 cells [4], constant pH and constant energy levels were observed in 1D ^{31}P NMR spectra of PC-3VEGFc2 cells combined with HUVECs on ECM gel, despite continuous hypoxia. Phosphocreatine (PCr) levels increased under hypoxia for PC-3 cells as well as for PC-3VEGFc2 cells (**Fig. 5**).

Discussion: Under hypoxia, PC-3 cells overexpressing VEGF combined with HUVECs on ECM gel exhibited increased invasion compared to wild-type PC-3 cells under the same conditions. These results suggest that cancer cells overexpressing VEGF in the presence of endothelial cells may be capable of increased invasion in regions of the tumor characterized by cyclic or chronic hypoxia. These findings may explain, in part, the increased rates of metastases observed in patients with cancers secreting high levels of VEGF. Irrespective of VEGF expression, intracellular PCr increased under hypoxia, while ATP levels remained constant. These findings suggest a shutdown of energy consuming processes such as proliferation in favor of maintaining ATP levels [5].

References:

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Acknowledgements:

This work was supported by NIH Grant 2 RO1 CA73850. We thank Genentech, CA, for providing full-length VEGF cDNA, Dr. V.P. Chacko, Mrs. P. Kollars and Mrs. Y. Mironchik for their technical assistance.