VEGF Overexpression Combined with Hypoxia is Associated with Increased Invasion and Changed Cellular Metabolism in a Human Prostate Cancer Cell Line

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Introduction: Hypoxia, increased tCho (total choline) levels and increased expression of the potent permeability factor, vascular endothelial growth factor (VEGF), in tumors have been linked to increased metastasis [1, 2]. VEGF expression is stimulated under hypoxia through the binding of HIF-1 to the hypoxia response element in the promoter region of VEGF [3]. Also, VEGF is frequently upregulated in cancer cells [3]. We generated PC-3VEGFc2, stably-transfected PC-3 cells overexpressing $VEGF_{165}$. Using PC-3VEGFc2 and parental PC-3 cells in our MR-compatible invasion assay [4], we examined the effects of VEGF overexpression on the invasion and metabolism of these cancer cells co-cultured with human umblical vein endothelial cells (HUVECs) under oxygenation or hypoxia.

Material and Methods: PC-3 cells (ATCC) were originally derived from a bone metastasis of a prostate cancer patient. PC-3VEGFc2 cells 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. About 16 h prior to all MR experiments, $5x10^4$ HUVECs (Clonetics) were added in a chamber on the surface of polymerized ECM gel. Within that time, HUVECs formed a lumen-like structure on the ECM gel. Adherently grown cancer cells were layered on either side of the ECM gel chamber in a customized 10-mm NMR tube and perfused with cell culture medium. The sample temperature was kept at 37°C and the oxygen tension 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 over the entire sample by 1D ^{1}H MRS and 1D ^{31}P MRS and along the sample in 310 µm thick slices by 1D ^{1}H CSI.

Results:

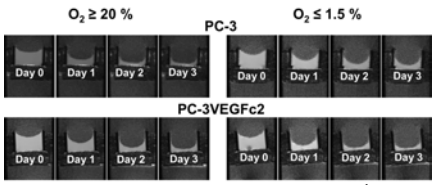


Figure 1: Representative T_1-weighted ^{1}H MR images of PC-3 and PC-3VEGFc2 cells, both combined with HUVECs on ECM gel demonstrating similar rates of degradation of ECM gel under oxygenation, and increased degradation of ECM gel under hypoxia by PC-3VEGFc2 cells compared to PC-3 cells.

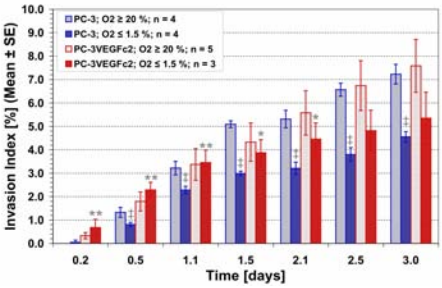


Figure 2: Invasion Index of PC-3 cells compared to the VEGF-overexpressing clone PC-3VEGFc2, in the presence of HUVECs on ECM gel, under well-oxygenated ($O_2 \geq 20$ %) and ($O_2 \leq 1.5$ %) hypoxic conditions.

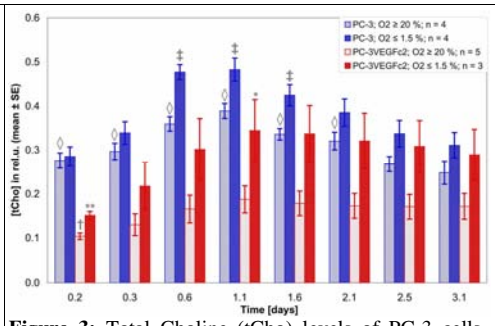


Figure 3: Total Choline (tCho) levels of PC-3 cells compared to PC-3VEGFc2 cells, in the presence of HUVECs on ECM gel, under well-oxygenated ($O_2 \geq 20$ %) and ($O_2 \leq 1.5$ %) hypoxic conditions. Data were obtained from global 1D ^{1}H MR spectra.

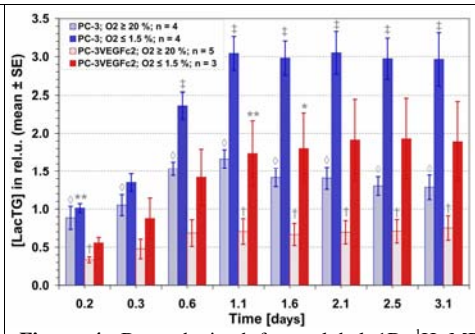
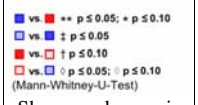


Figure 4: Data obtained from global 1D ^{1}H MR spectra demonstrated that in the presence of HUVECs on ECM gel, intracellular LacTG (Lactate + Triglycerides) levels of PC3 cells were significantly higher compared to PC-3VEGFc2 cells.



Shown above is the legend for markers and their corresponding significance levels from the statistical analysis of the data in Fig. 2 – 4.

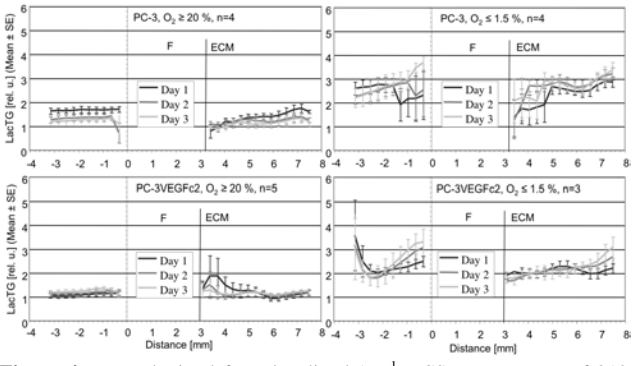


Figure 4: Data obtained from localized 1D ^{1}H CSI MR spectra of 310 µm thick slices along the sample. In the presence of HUVECs on ECM gel, hypoxia increased intracellular LacTG independent of the VEGF expression status.

Parental PC-3 cells, PC-3pCR3.1, PC-3VEGFc2, and a 2nd VEGF-overexpressing clone of PC-3 exhibited the same invasiveness under control conditions (data not shown). VEGF overexpression did not alter the invasiveness of PC-3 cells under well-oxygenated conditions (Fig. 1, Fig. 2). However, VEGF overexpression reversed the decrease of invasion as a result of hypoxia in parental PC-3 cells, leading to hypoxic PC-3VEGFc2 cell invasion values comparable to those of well-oxygenated cancer cells (Fig.2). VEGF overexpression did not influence the energy status or intracellular pH of PC-3 cells (data not shown). VEGF overexpression lowered tCho levels significantly in oxygenated cancer cells, an effect not apparent in hypoxic cancer cells (Fig. 3). Hypoxia tended to increase – for some time points significantly – tCho levels in the cancer cells irrespective of their VEGF expression status (Fig. 3). Changes of tCho levels over time could be explained in part by phosphocholine changes (data not shown). Intracellular LacTG levels were significantly decreased by VEGF overexpression (Fig.4). LacTG levels increased significantly under hypoxia independent of VEGF expression status (Fig. 4). These LacTG changes were primarily due to lower intracellular triglyceride levels as intracellular lactate levels were independent of the VEGF expression status and increased only marginally under hypoxia (data not shown). Localized 1D ^{1}H CSI MR spectra reflected the changes of LacTG levels observed in global 1D ^{1}H MR spectra (Fig. 5). Compared to cancer cells distant from the ECM, LacTG levels in cancer cells in the presence of HUVECs at the invading front did not change significantly (Fig. 5).

Discussion: Our results suggest that VEGF overexpression of cancer cells in the presence of endothelial cells may increase cancer cell invasion in hypoxic tumor regions. Hypoxia increased intracellular tCho and LacTG levels irrespective of VEGF expression levels. Our results may in part explain the increased occurrence of metastasis observed in patients with cancers secreting high levels of VEGF. Our data also suggest that high tCho in tumors may be related to hypoxia.

References: [1] E Ackerstaff *et al.* J Cell Biochem, *90:* 525-33, 2003. [2] LM Ellis *et al.* Eur J Cancer, *32A:* 2451-60, 1996. [3] GU Dachs *et al.* Eur J Cancer, *36:* 1649-60, 2000. [4] U Pilatus *et al.* Neoplasia, *2:* 273-9, 2000. **Acknowledgements:** This work was supported by NIH Grant 2RO1CA73850. We thank Genentech, CA, for providing $VEGF_{165}$ cDNA, Dr. V.P. Chacko, Mr. G. Cromwell, Ms. P. Kollars and Ms. Y. Mironchik for their technical assistance, Dr. D. Shungu and Ms. X. Mao for XsOsNMR.