VEGF overexpression in MCF-7 breast cancer cells increases invasion and lactate production

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Introduction: The progressive growth and metastasis of breast and other cancers are angiogenesis-dependent processes (1). Vascular endothelial growth factor (VEGF) is a potent angiogenic factor in tumors, including endocrine-responsive tumors. VEGF expression is elevated in human breast tumors and negatively influences survival (2). In humans $VEGF_{121}$ and $VEGF_{165}$ are two predominant isoforms known to play an important role in neovascularization and tumor progression (3). VEGF produced by breast carcinoma cells is known to stimulate angiogenesis through paracrine mechanisms in tumor endothelial cells. VEGF receptors are also found in breast cancer cells but the autocrine role of VEGF in breast cancer cells is less well known. In this study, we have investigated the effect of $VEGF_{165}$ in the invasion and metabolism of MCF-7 cells using an MR compatible cell perfusion-invasion assay, the Metabolic Boyden Chamber.

Material and Methods: We generated MCF-7 human breast carcinoma cells stably overexpressing $VEGF_{165}$ cells (VEGF MCF-7) using the construct pHuVEGF.21 (Genentech, CA) cloned into the expression vector pCR3.1; empty vector-transfected MCF-7 cells and wild type MCF-7 served as controls. For the MR cell perfusion studies, three days prior to the experiments, cells were seeded on Biosilon beads (Nunc, Denmark) beads at a cell density of 1.5 $X10^6$ cells per 0.5 ml of microcarriers in non–cell culture petri dishes and grown adherently to these beads to approximately 70% confluency. The sample consisted of different layers containing filter material, cell covered beads, a home-built chamber filled with extracellular matrix (Matrigel®), and perfluorocarbon-doped alginate beads to measure oxygenation in the 10mm NMR tube, as previously described (4). A schematic of the sample preparation is shown in Fig. 1a.

Results: Fig.1b shows representative ^{1}H MR images of Matrigel® degradation acquired over 48 h that demonstrate a significant increase of Matrigel® degradation VEGF MCF-7 cells. In contrast, and consistent with their poorly invasive and metastatic phenotype, control cells showed negligible degradation of Matrigel®. For statistical analysis data from EV MCF-7 (n=2) and wild type MCF-7 cells (n=1) were pooled together. Quantitative time-dependent invasion indices I(t) obtained from diffusion weighted 1D ^{1}H profiles of intracellular water showed a significant increase in invasion by VEGF MCF-7 cells compared to control cells ($p < 0.05$) (Fig. 1c).

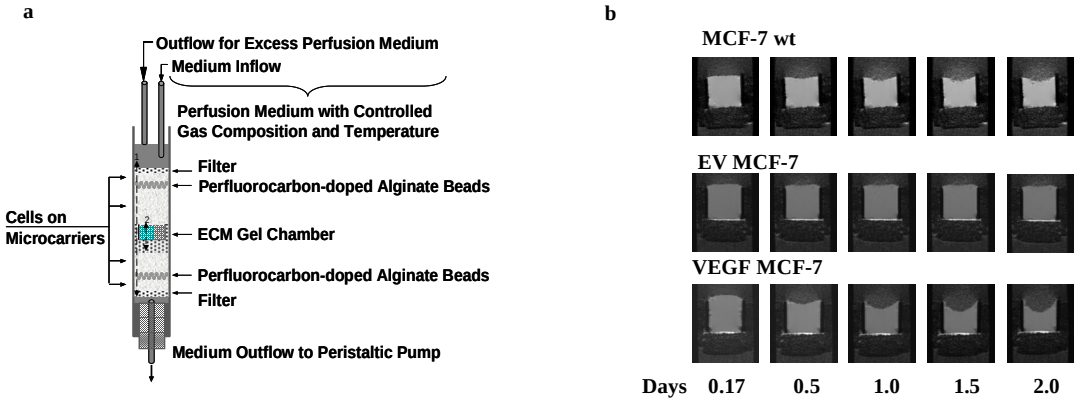

Figure 1: (a) Schematic of the sample preparation for perfusion studies. (b) Representative T_1-weighted ^{1}H MR images showing significant degradation of Matrigel® by VEGF MCF-7 cells while EV MCF-7 and wild type MCF-7 cells showed negligible degradation over similar time points.

A significant increase in lactate (Lac) was observed in VEGF MCF-7 cells compared to control MCF-7 cells ($p < 0.06$) but we did not observe a significant difference in total Cho or phosphocholine levels. Data quantifying lactate in VEGF MCF-7 and control MCF-7 cells over the course of two days are summarized in Fig. 2 and demonstrate increased Lac with $VEGF_{165}$ overexpression. There were no differences in other invasion related genes such as uPAR and MMP-2 between VEGF MCF-7 and control MCF-7 cells as measured using qPCR.

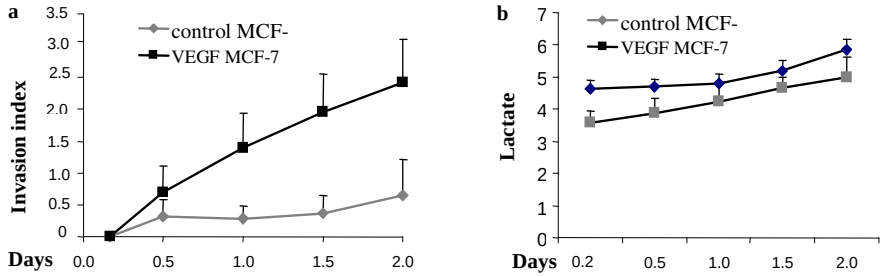

Figure 2: (a) Quantitative time-dependent invasion indices I(t) were obtained from intracellular diffusion-weighted water profiles at various time points. Values are mean ± SD (n = 3; * $p < 0.05$). (b) Quantification of ^{1}H spectra identified significant differences in the amount of lactate between VEGF MCF-7 and control MCF-7 cells (n=3, $p < 0.06$).

Discussion: The increased lactate production in VEGF MCF-7 cells is consistent with their increased malignant behavior since increased glycolysis and acidic extracellular pH are associated with a more malignant phenotype (5). The increased invasion by VEGF MCF-7 cells of Matrigel® may have occurred from the interplay between VEGF and ECM components since VEGF is known to induce mitogenic effects and migratory response in breast cancer cells (6). It has been shown that both estrogen and VEGF regulate a similar subset of genes that promote breast cancer progression (7). Up-regulation of VEGF in estrogen-dependent breast cancers also contributes to the acquisition of estrogen-independent cancer growth by stimulating tumor angiogenesis and progression (8). These data support the possibility that, in addition to its known paracrine effects, VEGF can increase invasion and alter metabolism of cancer cells through autocrine signaling, providing additional reasons for targeting this cytokine.

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