Lymphatic Endothelial cells Enhances Prostate Cancer Cells Invasion of Extracellular Matrix
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Introduction
The presence of lymph node metastasis is of major prognostic significance for many cancers, including prostate cancer [1]. Clinicopathological studies have suggested that lymphatic vessels serve as the primary route for the metastatic spread of tumor cells to regional lymph nodes. Recent studies in animal models have provided convincing evidence that tumor lymphangiogenesis facilitates lymphatic metastasis [2]. However, it is not clear how tumor-associated lymphangiogenesis is regulated and the microenvironment factors required that affect the invasion of cancer cells into lymphatic vessels. Here we have investigated the role of lymphatic endothelial cells-prostate-cancer cell interaction in invasion and degradation of the extracellular matrix (ECM) in our MR compatible cell perfusion assay, and also determined the associated metabolic changes.

Material and Methods
Experiments were performed using human prostate cancer cell lines PC-3 (ATCC, Manassas, VA), maintained in RPMI medium and human dermal lymphatic microvascular endothelial cells (HMVECs) obtained from Lonza (Walkersville, MD), maintained in EGM-2 MV medium (Lonza). Before each MR experiment, 2.5x10^6 PC-3 cells were seeded on 0.5 ml of Plastic Plus beads (Sotolhill, Ann Harbor, MI) and grown for 3 days. Experiments were carried out either with PC-3 cells alone plated on ECM chamber or with HMVECs layered between the PC-3 cells and the ECM. For MR experiments investigating lymphatic cells -cancer cell interaction, 5 X 10^5 HMVECs were seeded on ECM gel contained in a chamber overnight before the MR experiment. This time interval allowed HMVECs to attach to the ECM gel and form a branching tubular network. A detailed description of the MR compatible cell perfusion system can be found in Ackerstaff et al. [3]. MR data were acquired on a 9.4 T MR spectrometer (Bruker, Billerica, MA) every 12 h over a period of 2 days. T1-weighted 1H MR images were obtained either with PC-3 cells alone or with HMVECs. Signals from total intracellular water, which represents intracellular cellular water, is directly proportional to the number of cells. The invasion index I(t) at time t was calculated as follows:

\[ I(t) = \frac{I_{p,7mm}(t)}{I_{p}(t)} - \frac{I_{p,7mm}(1)}{I_{p}(1)} \]

where \( I_{p,7mm}(t) \) is the integral value of the signal at time t, obtained by integrating intracellular water signal over a 7-mm region starting at the base of the ECM chamber, and \( I_{p}(t) \) is the integral for the entire diffusion-weighted profile at time t. The first contact of cancer cells with the ECM gel during the loading of the sample was defined as the zero time point, and t1 defines the first MR data set acquired after loading. Intracellular metabolite levels including total choline (tCho), i.e., signals from phosphocholine (PC) + glycerophosphocholine (GPC) + free choline, creatine/phosphocreatine (Cr/PCr), and lipids were also detected in proton spectra obtained from perfused PC-3 cells under normoxia. Signals from energy metabolites, pH, and the phospholipid metabolites PC and PE were obtained from global 1D 31P MR spectra. All MR data were processed using XsOs MR Software.

Results and Discussion
Figure 1a shows representative 1H MR images of ECM gel degradation by parental PC-3 cells alone or and PC-3 cells in the presence of HMVECs acquired over 48 h. PC-3 cells degraded the ECM under both conditions. However ECM degradation significantly increased when HMVECs were layered between the ECM and the PC-3 cancer cells. Figure 1b shows quantitative time-dependent invasion indices I(t) obtained from diffusion weighted 1D 1H profiles of intracellular water acquired at 48 h from experiments acquired with or without HMVECs in the presence of the ECM. There was a significant increase in the invasion index in the presence of HMVECs compared to PC-3 cells alone (p <0.08). Figure 1c shows representative 1H and 31P spectra obtained from perfused PC-3 cells under normoxia. Signals from total choline (tCho), Cr, glutamate/glutamine (gls) and a prominent signal consisted of lipids and lactate was detected in proton spectra. Signals from phosphoethanolamine (PE), phosphocholine (PC), inorganic phosphate (Pi), and NTPs were detected in 31P spectra. No significant differences were observed in these metabolites for experiments performed with PC-3 cells alone or with PC-3 cells in the presence of HMVECs.

The enhanced degradation of ECM by PC-3 cells in the presence of HMVECs indicates that the interaction between lymphatic endothelial cells and prostate cancer cells plays a critical role in lymphatic metastasis. Since tumors are also characterized by hypoxia and acidic extracellular pH, our ongoing studies with the MR-compatible cell perfusion system will allow us to investigate the influence of these physiological conditions on lymphatic endothelial cell-prostate cancer cell interactions and invasion and metastasis.

References: (1) Sleeman J, Schmid A, Thiele W. Semin Cancer Biol. 2009;19:2009;107:1053-62. (2) Veikkoila T, Jussila L, Makinen T et al EMBO J 2001;20:1223-1231. (3) Ackerstaff E, Gimi B, Artemov D, Bhujwalla ZM Neoplasia. 2007; 9: 222-235. Acknowledgements: This research was supported by NIH R01CA73850 and P50CA103175. We thank Dr. Dikoma Shungu and X. Mao for the XsOs software and Dr. Ellen Ackerstaff for useful discussions.