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 (Solohill, 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, $5 X 10^4$ 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 ^{1}H MR imaging was performed to evaluate the sample preparation, to visualize the geometry of the ECM gel, and to detect changes in its integrity due to invasion and degradation. One-dimensional (1D) ^{1}H MR profiles of intracellular water were acquired along the length (z-axis) of the sample by diffusion- weighted (DW) MRI. These profiles were used to derive an invasion index by quantifying the number of cells invading into the ECM, as the signal from slow-diffusion water, which represents intracellular water, is directly proportional to the number of cells. The invasion index I(t) at time t was calculated as follows:

$$It = I_{p,7mm}(t)/I_p(t) - I_{p,7mm}(t1)/I_p(t1)$$

where Ip,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 gel chamber, and Ip(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 derived from unlocalized DW ^{1}H MR spectra. Signals from energy metabolites, pH, and the phospholipid metabolites PC and PE were obtained from global 1D ^{31}P MR spectra. All MR data were processed using XsOs MR Software.

Results and Discussion

Figure 1a shows representative ^{1}H 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 ^{1}H profiles of intracellular water acquired at 48 h from experiments acquired with or without HMVECs present on 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 ^{1}H and ^{31}P spectra obtained from perfused PC-3 cells under normoxia. Signals from total choline (tcho) Cr, glutamate/glutamine (glx) 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 ^{31}P 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 .

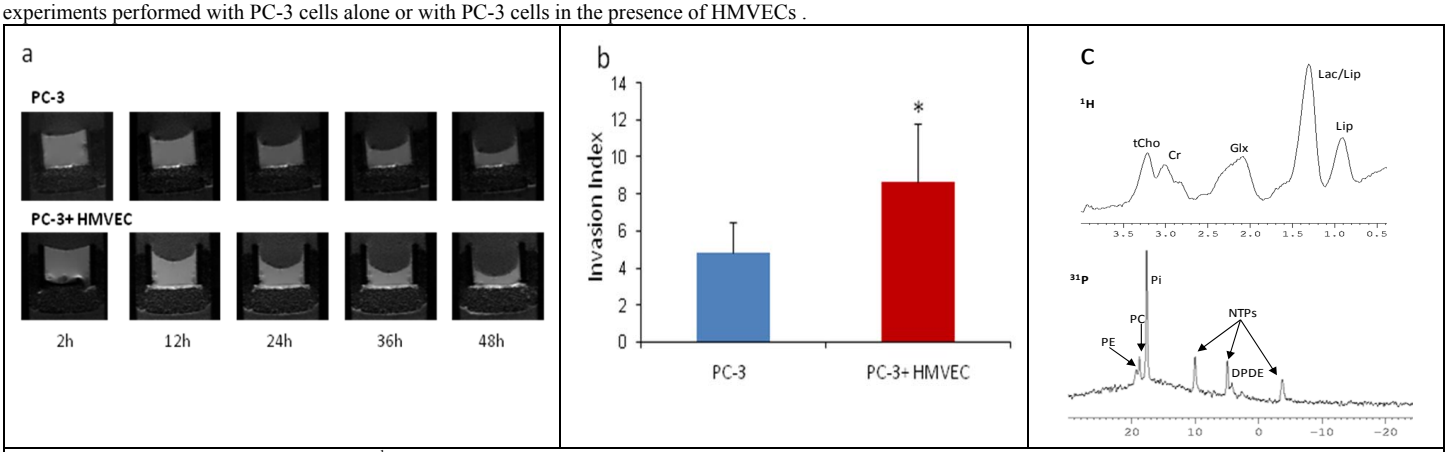

Figure 1: (a) Representative T_1-weighted ^{1}H MR images at the various time point zoomed into the region with the ECM chamber, showing degradation of ECM gel under normoxia. (b) Invasion index obtained from intracellular water signal at 48 h for PC-3 cells and for PC-3 cells in the presence of HMVECs (*$p<0.08$) under normoxia. Values are Mean ± SD. (c) Representative ^{1}H and ^{31}P MR spectra obtained from perfused cells at 24 h under normoxia.

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) Veikkola 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.