Role of Lymphatic Endothelial Cells in Prostate Cancer Cell Invasion in Tumor Microenvironments

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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 microenvironmental factors that affect the invasion of cancer cells into lymphatic vessels. Since hypoxia is a characteristic feature of the tumor microenvironment, 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 under normoxic and hypoxic conditions, and determined the associated metabolic changes.

Material and Methods: Experiments were performed using the human prostate cancer cell lines PC-3 and DU-145, and human dermal lymphatic microvascular endothelial cells (HMVEC-dly). Before each MR experiment, $2.5X10^6$ PC-3 and DU-145 cells were seeded on 0.5 ml of Plastic Plus beads and grown for 3 days and 4 days, respectively. Experiments were carried out with PC-3 or DU-145 cells plated on an ECM chamber or with HMVEC-dlys layered between the PC-3 or DU-145 cells and the ECM. For experiments investigating lymphatic cell-cancer cell interaction, 5 X 10^4 HMVEC-dly were seeded on ECM gel contained in a chamber overnight before the MR experiment. This time interval allowed HMVEC-dly to attach to the ECM gel and form a branching tubular network. MR data were acquired on a 9.4 T MR spectrometer every 12 h over a period of 2 days. T1-weighted ^{1}H MR imaging was performed to evaluate changes in ECM invasion and degradation. One-dimensional ^{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 described by us previously [3]. Intracellular metabolite levels were derived from unlocalized DW ^{1}H and ^{31}P MR spectra.

Results and Discussion: Figures 1a-b show representative ^{1}H MR images of ECM gel degradation by parental PC-3 or DU-145 cells alone or in the presence of HMVEC-dly, acquired over 48h under normoxic or hypoxic conditions. PC-3 cells degraded the ECM under both conditions. However ECM degradation significantly increased when HMVEC-dly were layered between the ECM and the PC-3 prostate cancer cells. DU-145 prostate cancer cells showed only moderately increased invasion in the presence of HMVEC-dly. **Figures 1c-d** show quantitative time-dependent invasion indices I(t) obtained from diffusion weighted 1D ^{1}H profiles of intracellular water acquired at various time points from experiments acquired with or without HMVEC-dly present on the ECM. There was a significant increase in the invasion index in the presence of HMVEC-dly compared to PC-3 cells alone ($p < 0.05$). Decreased invasion of ECM by PC-3 cells was observed under hypoxia ($p<0.05$), compared to normoxia. **Figures 2a-b** shows significant differences in basal level of both total choline (tCho) and phosphocholine (PC) levels between invasive PC-3 cells compared to the less invasive DU-145 cells ($p<0.05$). No significant differences were observed in these metabolites for experiments performed with each prostate cancer cells alone or in the presence of HMVEC-dlys. Significantly higher glycerophosphochline was observed in DU-145 cells compared to PC-3 cells (data not shown). Significant increase in lipid levels were observed in both PC-3 and DU-145 cells under hypoxia as shown in **Figures 2c-d.**

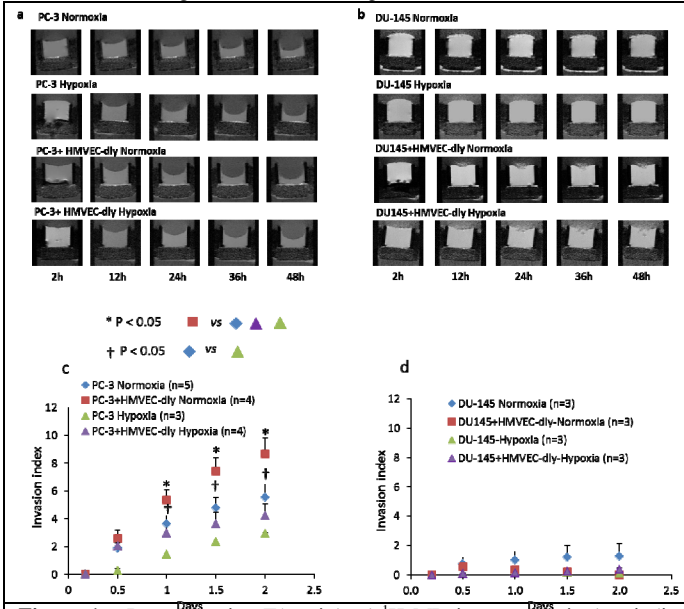

Figure 1: Representative T1-weighted ^{1}H MR images acquired at indicated time points of the ECM chamber region, showing degradation of ECM gel over a period of 48h by (a) PC-3 and (b) DU-145 prostate cancer cell lines with or without HMVEC-dly cells under normoxia or hypoxia. Invasion index obtained from intracellular water signal at various time points in (c) PC-3 cells and (d) DU-145 prostate cancer cells in the presence/absence of HMVEC-dly under normoxia or hypoxia. Values are Mean ± SEM.

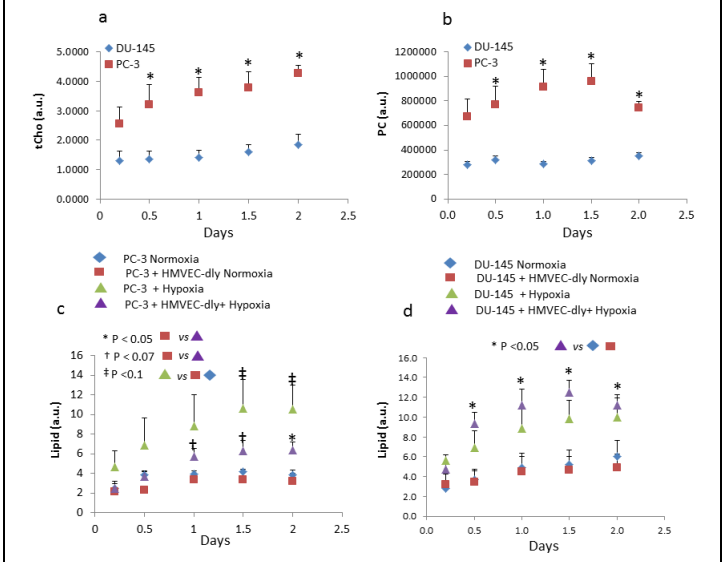

Figure 2: Significantly higher (a) tCho obtained from ^{1}H MR spectra and (b) PC levels obtained from ^{31}P MR spectra in PC-3 compared to DU-145 prostate cancer cells. Values are Mean ± SEM; * $p < 0.05$. A significant increase of lipid levels under hypoxia was observed in (c) PC-3 and (d) DU-145 prostate cancer cells over a period of 48h. Values are Mean ± SEM.

Although hypoxia reduced the invasion of PC-3 prostate cancer cells, the presence of lymphatic endothelial cells alleviated this reduction in invasion. The enhanced degradation of ECM by PC-3 cells in the presence of HMVEC-dly indicates that the interaction between HMVEC-dlys and prostate cancer cells play a critical role in lymphatic metastasis. The presence of HMVEC-dly may significantly influence metastatic potential in tumor progression. The increase in lipids may represent an adaptive metabolic response to hypoxia.

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.