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-cancer cell interaction, 5X10^4 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 1H MR imaging was performed to evaluate changes in ECM invasion and degradation. One-dimensional 1H 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 1H and 31P MR spectra.

Results and Discussion: Figures 1a-b show representative 1H 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-dlys 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 1H 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 glycerophosphocholine 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.

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-dlys indicates that the interaction between HMVEC-dlys and prostate cancer cells play a critical role in lymphatic metastasis. The presence of HMVEC-dlys may significantly influence metastatic potential in tumor progression. The increase in lipids may represent an adaptive metabolic response to hypoxia.