Metabolic Boyden Chamber Detects Significant Differences in Invasion for Metastatic and Nonmetastatic Prostate Cancer Cells.

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INTRODUCTION: The physiological milieu within solid tumors can influence invasion and metastasis [1-3]. To determine the impact of the physiological environment on cancer cell invasion it is necessary to measure invasion during well controlled modulation of the physiological environment. Current methods cannot measure invasion dynamically. Recently, we demonstrated that magnetic resonance imaging can be used to monitor, dynamically, cancer cell invasion into the matrigel layer of standard invasion chambers [4]. Here we have developed an invasion assay ('Metabolic Boyden Chamber') which combines this capability with the properties of our isolated cell perfusion system enabling us to control environmental conditions during long term MR experiments of perfused cells. Using this assay we performed experiments with prostate cancer cell lines with different invasive characteristics. The results showed invasion into, and degradation of the matrigel layer, by the highly invasive/metastatic line while no significant changes were observed for the less invasive/metastatic cell line.

METHODS: Experiments were performed with the highly invasive rat prostate cancer cell line MatLyLu and the less invasive human prostate cancer cell line DU-145. Immobilized cells, adherently grown on polystyrene beads, were transferred into a 10 mm NMR tube. To obtain information on cell migration and invasion, a structure consisting of a layer of matrigel in a filter cup was placed between cell covered beads. The sample was perfused continuously and cell metabolism and growth were studied in each layer by 1D Chemical Shift Imaging. The profile of intracellular water along the z axis of the sample was obtained by diffusion weighted 1H NMR (Fig. 1) [5], using gradient pulses of 3 ms, gradient of 18 G/cm, diffusion weighting time of 100 ms. Cell invasion was detected from changes in the profile with time, reflecting invasion of cells from polystyrene beads into the matrigel layer in the filter cup. Environmental conditions for cells in the chamber were controlled by perfusing with well defined media. Oxygen tension was adjusted by bubbling defined gas mixtures through the medium reservoir and monitored by localized 19F MR relaxometry of alginate beads doped with perfluorocarbons. Perfluorocarbons in the matrigel were used to determine oxygen tensions within the invasion chamber; doping of the matrigel layer with perfluorocarbons had an added advantage of providing information of the stability and position of matrigel over the time course of the experiment using ^{19}F MRI. Eight separate sets of experiments were performed for the MatLyLu line and five for the DU-145 cell line.

RESULTS: The region of the matrigel layer and the corresponding profile of the cellular water signal, obtained with a spatial resolution of 30 μm, is shown in Figure 1. We consistently observed that the matrigel layer was degraded by MatLyLu cells within 24 h, while it remained unchanged for DU-145 cells over the entire experiment (72 h). Figure 2 demonstrates determination of oxygen tensions in the Metabolic Boyden Chamber (MBC). Cell invasion into the matrigel layer of the MBC for the two prostate cancer cell lines is shown in Figure 3. Invasion of MatLyLu cells is evident from the profiles by a significant increase in cell density in the vicinity of the matrigel/microcarrier border compared to DU-145 cells. We obtained a quantitative index of cell invasion (I) by integrating the area of the diffusion weighted profile over a 5 mm region, extending from the base of the filter cup and including the matrigel layer. This area was divided by the entire cellular profile to correct for cell growth.

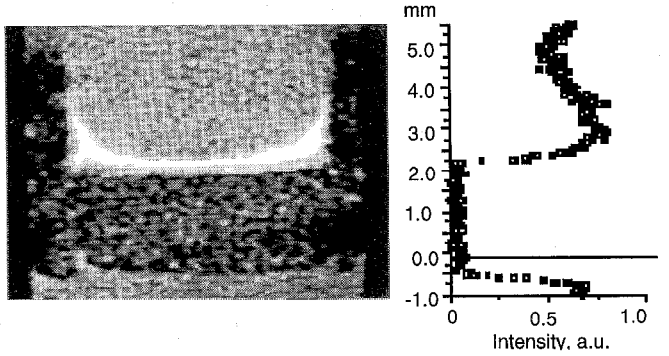

Fig.1: T1 weighted image of the invasion chamber and corresponding diffusion weighted profile (on the right).

Results averaged for the 8 MatLyLu experiments and 5 DU-145 experiments are shown in Figure 4. A significant difference ($p < 0.01$, paired t-test) was observed for I values at 24 and 48 h for the MatLyLu line but not for the DU-145 line. We have established that the technique shows reproducible differences in invasion. Studies are currently underway to probe the effect of alterations in oxygen tensions on the Invasion Index.

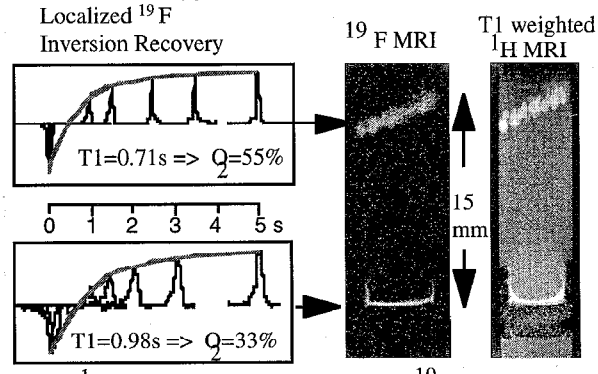

Fig. 2: ^{1}H MR image of the sample (right), ^{19}F MR image (center) and the inversion recovery of the ^{19}F signal of the perfluorocarbons at the upper layer and at the Matrigel layer of the sample (left).

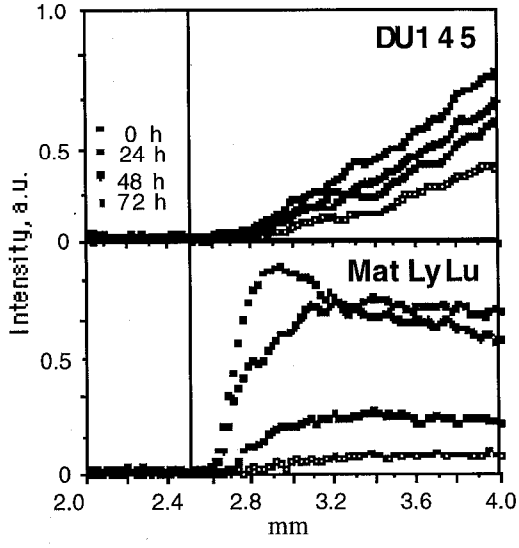

Fig. 3: Profiles of diffusion weighted water for DU-145 and MatLyLu cells. The line marks the lower border of the matrigel layer.

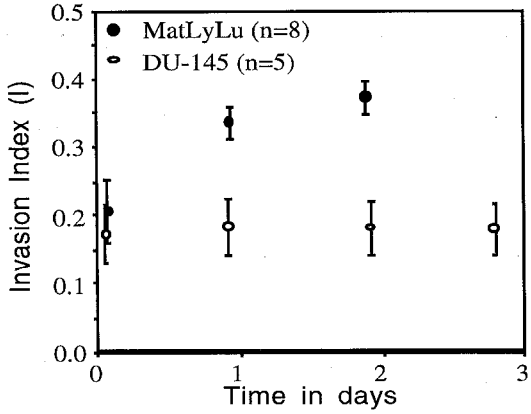

Fig. 4: Invasion Index (I) for the highly invasive MatLyLu line and the less invasive DU-145 line. Values are Mean ± 1 S.E.M.

References: 1.Young, S..D. et al., PNAS, 85, 1988; 2. Rozhin, J.,et al., Cancer Res. 54,1994; 3.Schwickert, G., et al., Cancer Res. 55, 1995; 4. Artemov, D. et al., Mag. Res. Med. (in press).; 5. van Zijl, P. et al., P.N.A.S., 88, 1991.

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