Hypoxia increases endothelial cell invasion and migration

B. Gimi[1], N. Mori[1], Z. M. Bhujwalla[1]

[1]The Johns Hopkins University School of Medicine, Baltimore, MD, United States

Introduction – Hypoxia is known to stimulate angiogenesis in tissue and is a common feature in tumors. Noninvasive dynamic studies visualizing the invasion and migration of endothelial cells in response to cancer cells or physiological environments characteristic of tumors is important to understand neovascularization of tumors. Here we have designed and used a non-invasive assay to study human endothelial cell invasion and motility in response to hypoxia.

Methods - A schematic of the assay is shown in Fig. 1. HUVECs were labeled with a superparamagnetic iron-oxide contrast agent (Feridex®, 9 μg per ml of EGM-2 medium). Chambers were maintained under normoxia or hypoxia (< 1% oxygenation) to observe the effect of oxygenation on HUVEC invasion and motility. T_2-weighted spin echo MR images of the chambers were obtained at the 24 h time point with the following acquisition parameters: field-of-view = 1.6 cm, acquisition matrix = 256 x 256, slice thickness = 0.5 mm, echo time TE = 60 ms, repetition time TR = 617 ms, and number of averages = 2. MRI was performed on a 500 MHz (11.74 T) wide-bore imaging system with a Bruker Avance™ spectrometer equipped with triple-axis gradients. The MR images were adaptively thresholded and binarized using ImageJ and the % area fraction of hypointensity, indicating HUVEC presence, was measured. The % fractional area values were tested for significance with a two-sided Wilcoxon rank sum test; $p < 0.05$ was considered significant.

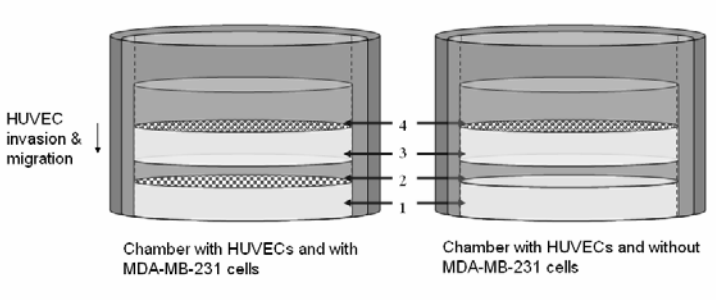

Fig. 1: Schematic of chambers: Millipore 24-well tissue culture inserts with 0.4 μm membrane pore size were layered as follows 1) 120 μl of ECM gel 2) Seed layer with 1.5 x 10^5 MDA-MB231 cancer cells (no cells in controls) 3) 100 μl ECM gel 4) 1.5 x 10^5 HUVECs labeled with iron oxide.

Results

We have previously shown that under normoxia and in the presence of MDA-MB-231 breast cancer cells, HUVECs increasingly invaded and migrated into the ECM gel toward the cancer cells over 120 h of observation, while no significant increase in invasion and motility was observed in the absence of cancer cells [1]. We report here that under hypoxia, HUVECs show increased invasion and migration into the ECM gel regardless of the presence of MDA-MB-231 cells. The effect of invasion and migration was quantified by calculating the % fractional area of HUVECs in the seed layer (indicated as layer "2" in Fig. 1). The % fractional area of HUVEC in the seed layer for chambers with MDA-MB-231 cells present was 7.11 % ±2.27 under normoxia (n = 6) and 13.93 % ±7.1 under hypoxia (n = 8). The % fractional area of HUVECs in the seed layer without cancer cells was 1.16 % ±1.77 under normoxia (n = 6) and 13.96 % ±7.2 under hypoxia (n = 8). HUVEC presence in the seed layer as observed with MRI was previously cross-validated in chambers under normoxia, with fluorescence staining of the endothelial cell specific monoclonal antibody CD31, and Prussian blue staining of iron content [1].

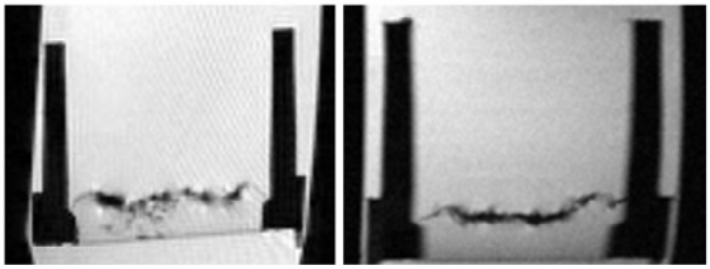

Fig 2: Coronal images of representative chambers with labeled HUVEC and no cancer cells in hypoxia (A) and under normoxia (B) at the 24 h time point. The HUVEC are seen penetrating the ECM under hypoxic conditions while no such penetration was observed under normoxia.

Discussion and Conclusions

While the presence of cancer cells increased the invasion and migration of HUVEC under normoxic conditions, under hypoxic conditions HUVEC invasion and migration was similar with or without MDA-MB-231 breast cancer cells. These data confirm that, even in the absence of cancer cells, hypoxia acts as a potent stimulus for invasion and migration of HUVEC. The non-invasive 3D MRI assay described can be used to characterize the role of other factors in the angiogenic cascade, to target key pathways for anti-angiogenic therapy, and to serve as a model system for a range of conditions involving aberrant angiogenesis.

Reference: 1. Gimi B, Mori N, Ackerstaff E, Frost EE, Bulte JWM, and Bhujwalla ZM, "An assay to study endothelial cell and network response to paracrine factors secreted by MDA-MB-231 breast cancer cells, using a superparamagnetic T2 contrast agent," *ISMRM Workshop on Advances in Experimental and Clinical MR in Cancer Research*, Manchester, UK, October 2004.

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