# Cancer cells induce lymphatic endothelial cell migration

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## Introduction

The 5-year survival rate from breast cancer drops from 96% to 75% with regional spread, and to 20% with distant spread with axillary lymph node-positive patients more likely to develop distant metastasis. The degree of lymph node and lung metastases has been shown to be highly correlated with the extent of tumor lymphangiogenesis in breast cancer [1]. Therefore the impact of both induction as well as inhibition of lymphangiogenesis is of significant interest. Vascular endothelial growth factors C (VEGF-C) and D (VEGF-D) are known to induce growth of lymphatic vessels through the activation of VEGF receptor-3 localized on the surface of lymphatic endothelial cells [2, 3]. While several studies have investigated the interaction between vascular endothelial cells and cancer cells using in vitro systems, studies investigating the interaction between lymphatic endothelial cells and cancer cells are relatively few. 2D and 3D *in vitro* assays for the quantitative analysis of cell invasion and migration offer the opportunity to better understand mechanisms of the formation of new blood and lymphatic vessels, discover new anti-migratory drugs as well as obtain new insights into the nature of interactions between cancer and lymphatic endothelial cells [4]. Here we have used a novel *in vitro* assay based on non-invasive magnetic resonance (MR) imaging of lymphatic endothelial cell movement through a three-dimensional extracellular matrix (ECM) gel. Using this system, we wanted to determine if the presence of human breast cancer cells at some nearby but physically removed site (on the side the ECM gel) would promote the migration of lymphatic cells. In these experiments a layer of MDA-MB-231 cells was separated from human lymphatic endothelial cells by a ~300 μm thick layer of ECM. Movement of magnetically labeled lymphatic endothelial cells was then monitored in real time by MRI for up to 120 h, and cell migration was quantified.

## Materials and Methods

MDA-MB-231 cells were originally derived from the pleural effusion of a breast cancer patient [5] and grown as a monolayer in RPMI medium. Human Dermal Lymphatic Microvascular Endothelial Cells (HMVEC-dLy) were obtained from Cambrex and maintained in endothelial cell medium (EGM-2 MV). Prior to preparing the chamber assay HMVEC-dLy cells were labeled with iron oxide nanoparticles (4 μg/ml) (Feridex, Berlex Inc.) and 55 ng/ml poly-L-lysine [6]. The invasion chamber was prepared by using cell culture inserts with a 12 mm diameter and size of membrane pores of 0.4 μm (Millicell, Millipore). ECM gel (Matrigel) from Engelbereth Holm-Swarm sarcoma was used at a concentration of 4.4 mg/ml as a barrier between HMVEC-dLy and MDA-MB-231 cells which were layered at the bottom of the cell culture insert [7]. MR images were acquired on a 500-MHz (11.74 T) wide-bore MRI system with a Bruker Avance spectrometer equipped with triple-axis gradients. Coronal and axial images of the chambers were obtained using a T2-weighted (TE=60 msec), multi slice, spin-echo sequence. HMVEC-dLy cells were stained with the LYVE-1 antibody and imaged by fluorescence microscopy to confirm that the protrusions were formed by HMVEC-dLy cells [8].

## Results

Chambers with (experimental) and without (control) MDA-MB-231 cells were imaged at 24, 48, 72 h and in some cases up to 120 h. At 24 h, HMVEC-dLy cells appeared as a monolayer at the top of the ECM gel (Fig. 1c). At 48 h HMVEC-dLy cells in the presence of MDA-MB-231 cells exhibited a tendency to concentrate at the center of the chamber, and also tended to migrate toward the cancer cells while the control remained unchanged from 24 h. By 72 h the HMVEC-dLy cells in the presence of MDA-MB-231 cells were significantly concentrated toward the center of the chamber, and clearly extended toward the lower part of the chamber (Fig. 1d). The controls showed a slight concentration of HMVEC-dLy cells toward the center of the chamber, but no detectable movement downward. The quantitative analysis of MR images is presented in Fig 2 and demonstrates significant changes in the HMVEC-dLy cell movement by 72 h in the presence of cancer cells. Light microscopic analysis of cells near the top of the gel at 120 h showed large aggregates of HMVEC-dLy cells with extensive protrusions connecting the aggregates to the MDA-MB-231 cells (Fig. 3 a). The control samples exhibited some cell aggregation, but no protrusions were evident (Fig. 3b). Immunofluorescent LYVE-1 staining of HMVEC-dLy cells is shown in Fig 3 (c, d).

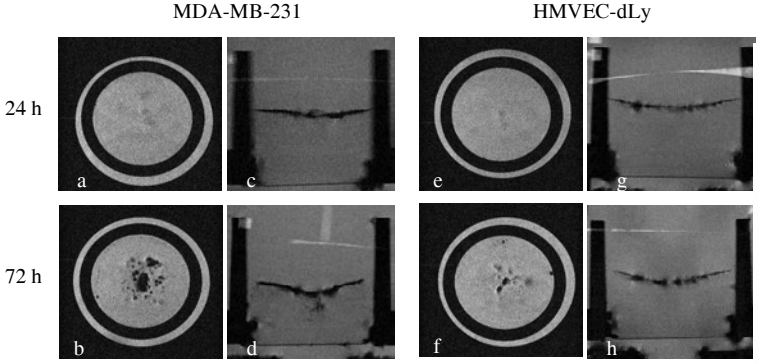


Figure 1. A panel of representative slices of axial (a, b, e, f) and coronal (c, d, g, h) MR images at 24 and 72 h of chamber with MDA-MB-231 cells and HMVEC- dLy cells (control).

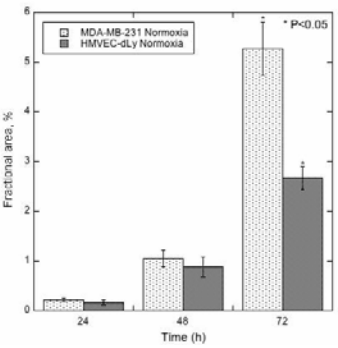


Figure 2. Fractional area (%) of labeled HMVEC-dLy cells from axial MR images vs time.

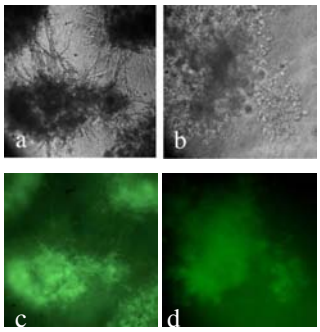


Figure 3. White light (a, b) and LYVE-1 stained fluorescent images (c, d) of chambers. a, c: cancer cells + HMVEC-dLy cells; b, d: HMVEC-dLy cells

## Discussion

MR images demonstrated a significant difference in the migration of HMVEC-dLy cells in the presence and absence of MDA-MB-231 cancer cells. Specifically the HMVEC-dLy cells were more motile and started to migrate through the ECM gel toward the cancer cells at 48 h. Additionally, in the presence of the cancer cells the HMVEC-dLy cells formed unusual aggregates that were interconnected strands of cells. While the importance of these structures is being investigated, our results suggest that HMVEC-dLy cells receive paracrine signals from the cancer cells causing them to migrate towards the cancer cells. We are currently using this noninvasive assay to understand the role of hypoxia in lymphangiogenesis.

## References

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