Cancer cell invasion analysis in ECM using *in vitro* models

Byoungkoo Lee[1], Jessica Konen[2], Songli Xu[3], Adam I. Marcus[4], Yi Jiang[5]

***Short Abstract* — Invasive cancer cells actively interact with the surrounding extracellular matrix fibers. Properly examining the invasive cancer cell behavior with ECM is necessary to more comprehensively understand cancer. We developed *in vitro* model to more quantitatively test the cancer cell behavior in ECM environments, using glioblastoma cultured in Matrigel and human non-small cell lung cancer cells cultured in collagen. We analyzed cell and fiber-bound bead motility as well as fiber alignment for various invasion conditions modifying key players along the signaling pathway: cdc42, Rho, FAK, LKB1. Active invasion conditions showed strong fiber pulling into the tumor and fiber realignment.**

I. Introduction

ONE main difficulty to treat cancer disease is due to invasion, in which cancer cells spread from their primary sites. *In vitro* tumor spheroid studies and transgenic mice studies have shown that invasive cancer cells actively remodel the surrounding ECM, and ECM alignment significantly influences cancer cell invasion [1]. Instead of focusing on cancer cells, we analyze the cells and ECM simultaneously, focusing on their interactions.

II. Results

To examine both cancer cell invasion and ECM dynamics together, we first cultured glioblastoma spheroids in Matrigel seeded with fluorescent beads, and tracked both cell and bead motions. Using the persistent random walk model [2], we analyzed cell and bead motility for different mechanotransduction signals: active cdc42, inactive cdc42, Rho inhibitor, and FAK inhibitor.

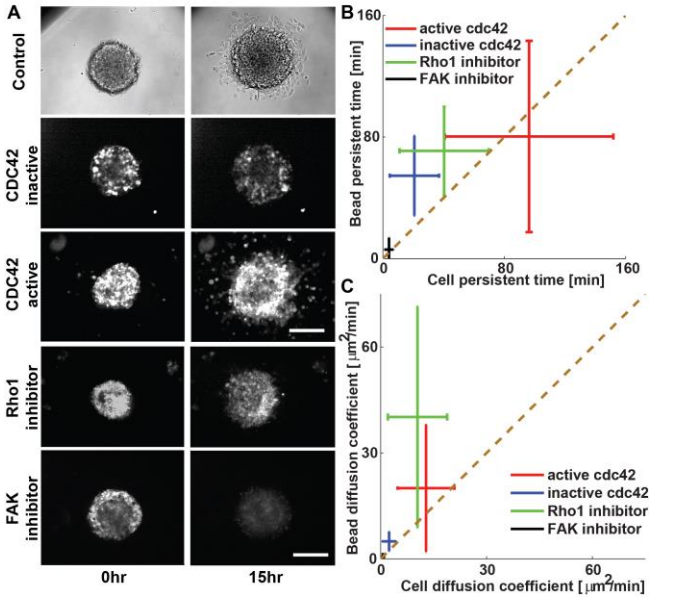

Figure 1: Glioblastoma spheroids in Matrigel. (A) Control and four different test cases (cdc42 inactive, cdc42 active, Rho inhibitor, FAK inhibitor) at 0 and 15 hours. Persistent time (B) and diffusion coefficient (C) for cell and bead motility data of four different test cases using a persistent random walk model.

In our experiments, Matrigel fiber-bound beads were pulled into the tumor spheroid before cancer cells actively invaded out. Active cdc42 cells (Fig.1) showed aggressive cell invasion and strong fiber pulling movement, while FAK inhibitor case showed neither cell migration nor fiber activity. Inactive cdc42 and Rho inhibitor cases showed more interesting cell-ECM traction in the absence of cell migration, and the rescue of migration in time.

To better visualize the ECM structural change as a result of cancer invasion, we cultured human non-small cell lung cancer (NSCLC) spheroids in collagen, and analyzed collagen fiber alignment for both LKB1+ and LKB1- spheroids. Using CT-FIRE (curvelet transform fiber extraction) [3] and an orientational order parameter [4], we developed a novel method to analyze fiber local alignment. LKB1- cells invade, resulting in increased fiber alignment. LKB+ cells do not invade, resulting in slightly decreased local alignment.

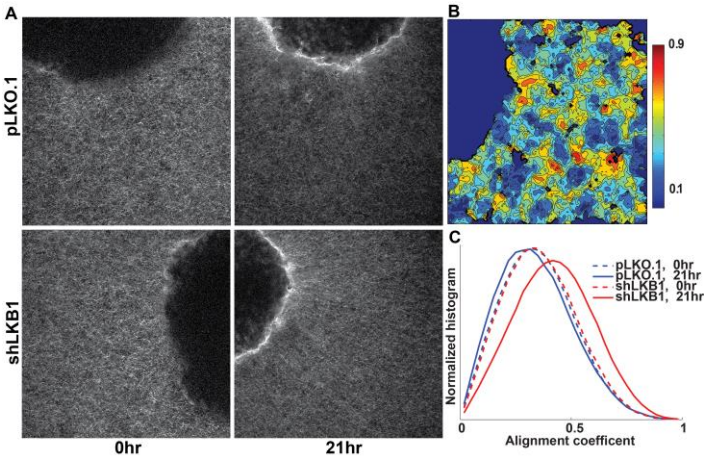

Figure 2: Human non-small cell lung cancer spheroids (H1299) in collagen gel. Control case (pLKO.1, LKB1+) and shRNA knocked down LKB1 case (shLKB1, LKB1-). (A) Confocal microscopy image at 0 and 21 hours. (B) Fiber alignment contour plot for the collagen image of shLKB1 at 21 hours. (C) Normalized fiber alignment histogram.

III. Conclusion

Glioblastoma cell and Matrigel-bound bead motility showed that invasive cells (active cdc42) "pull" the ECM into the tumor, as suggested by the strong correlation between cell and bead motility. Inhibiting different cell molecular signals altered both cell and bead motility. Inhibiting FAK showed almost block the cell and bead movement, while inactive cdc42 and Rho1 inhibitor showed moderate motility, which suggest that cell has multiple redundant signaling pathway via these signals. LKB1 inhibits FAK, and thus LKB1+ NSCLC did not show collagen fiber realignment, while LKB- NSCLC increased the fiber alignment. Our *in vitro* models provide alternative view on cancer invasion and help to better understand metastatic cancer in various ECM conditions.

References

[1] Provenzano PP, et al. (2006) Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC Med* **4**, 38.

[2] Potel MJ, Mackay SA. (1979) Preaggregative cell motion in *Dictyostelium*. *J Cell Sci* **36**, 281-309.

[3] Bredfeldt JS, et al. (2014) Computational segmentation of collagen fibers from second-harmonic generation images of breast cancer. *J Biomed Opt* **19**, 016007.

[4] Vader D, et al. (2009) Strain-induced alignment in collagen gels. *PLoS ONE* **4**, e5902.

[1,5]Department of Mathematics and Statistics, Georgia State University, [1]E-mail: blee37@gsu.edu, [5]E-mail: yjiang12@gsu.edu, [2]Caner Biology Graduate Program, Emory University, E-mail: jtepe@emory.edu, [3]Ceners for Disease Control and Prevention, [4]Department of Hematology and Oncology, Emory University, E-mail: aimarcu@emory.edu