2022

# Evaluation of Cell-Matrix Interactions in K14+ Leader Cells on CAF-Modulated Matrix

Trey P. Redman
*Virginia Commonwealth University*

Jessanne Y. Lichtenberg
*Virginia Commonwealth University*

Priscilla Y. Hwang
*Virginia Commonwealth University*

# Evaluation of Cell-Matrix Interactions in K14+ Leader Cells on CAF-Modulated Matrix

Trey P. Redman, Jessanne Y. Lichtenberg, Priscilla Y. Hwang

*Department of Biomedical Engineering, Virginia Commonwealth University*

## BACKGROUND

- Collective cell migration is the process by which clusters of cells migrate together as a cohesive unit. During collective migration, cells at the front edge are believed to lead this coordinated effort and are known as leader cells.
- The tumor microenvironment is a crucial factor influencing collective cell migration, specifically turning on leader cells so they know which direction to migrate.
- *In vivo* the tumor microenvironment is composed of various elements including activated cells, growth factors, cytokines, and extracellular matrix (ECM) (Fig. 1). Cancer associated fibroblasts (CAF) exist in the breast cancer tumor microenvironment and have been shown to regulate tumor metastases and development through remodeling stromal collagen and other ECM components [2].
- Work in our lab demonstrate that the collagen receptor, discoidin domain receptor 2 (DDR2), is responsible for changes in collagen fiber orientation *in vivo*, and without DDR2, CAFs lose their ability to lay down organized, aligned fibers that can promote cell invasion and metastasis (Fig. 2) [3].
- However, it is still largely unknown how CAF-modulated changes in matrix fiber orientation regulate signaling in leader cells that result in invasion.

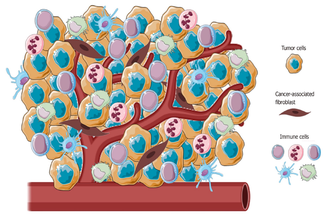


**Figure 1.** Diagram depicting the tumor microenvironment with tumor cells, CAFs, immune cells, and vasculature [1].

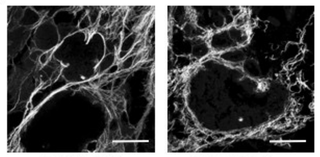


**Figure 2.** Collagen fiber orientation of tumor matrix from WT and FSP1Cre;DDR2$^{-/-}$ (CAF-modulated) mice [3].

## METHODOLOGY

**Tumor Decellularization**

Tumors were isolated from MMTV-PyMT (WT) and FSP1Cre; Ddr2$^{-/-}$; MMTV-PyMT (CAF-modulated) mice and decellularized. To decellularize the tumors, we chemically removed the cells, lyophilized the frozen decellularized extracellular matrix (dECM), and cryomiled it into a fine powder. The dECM was digested in pepsin and neutralized to form a gel. Glass bottom dishes were coated with the dECM overnight [4].

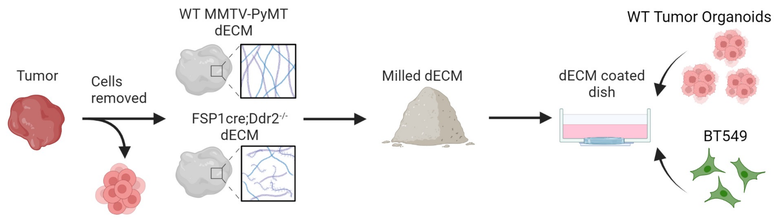


**Figure 3.** Schematic representation of the methods: tissue decellularization process and plating of tumor cells (created in Biorender).

**Cell Seeding and Immunofluorescence Staining**

Human breast cancer cell line, BT549 (positive for leader cell marker, K14) or WT MMTV-PyMT tumor organoids were cultured on dECM-coated glass bottom dishes. After cells were cultured for 24 hours, we performed immunofluorescence staining to identify focal adhesions (vinculin), actin cytoskeleton (phalloidin), and nuclei (DAPI). Cells were imaged using confocal microscopy (Zeiss LSM980). The focal adhesions and cell area were quantified using FIJI. Filopodia were analyzed using the open-source MATLAB script FiloDetect [5].

## OBJECTIVE & HYPOTHESIS

- The **objective** is to evaluate if and how tumor leader cells on CAF-modulated (FSP1Cre;Ddr2$^{-/-}$) matrix behave differently than on wild-type (WT) tumor matrix in order to determine how these cells are mechanically affected by the tumor microenvironment.
- We **hypothesize** that K14+ tumor leader cells will exhibit fewer cell-matrix interactions on CAF-modulated (FSP1Cre;Ddr2$^{-/-}$) matrix than on wild-type (WT) tumor matrix, as indicated by focal adhesion formation and actin stress fibers.

## RESULTS & DISCUSSION

**Leader cells are unable to form focal adhesions and aligned actin stress fibers on FSP1Cre;Ddr2$^{-/-}$ dECM.**

- BT549 cells form fewer focal adhesions on FSP1Cre;Ddr2$^{-/-}$ dECM compared to wild type dECM (Fig 4A-B). Percentage of cell area occupied by focal adhesions and focal adhesions per unit area are greater for cells on WT MMTV-PyMT dECM (Fig. 4E&G).
- Actin stress fibers are more prominent in BT549 cells on the WT MMTV-PyMT dECM (Fig. 5A).
- Cell area (Fig. 4A&D), focal adhesion area per cell (Fig. 4C), number of filopodia and length of filopodia (Fig. 5B-C) are similar for cells on both matrices.

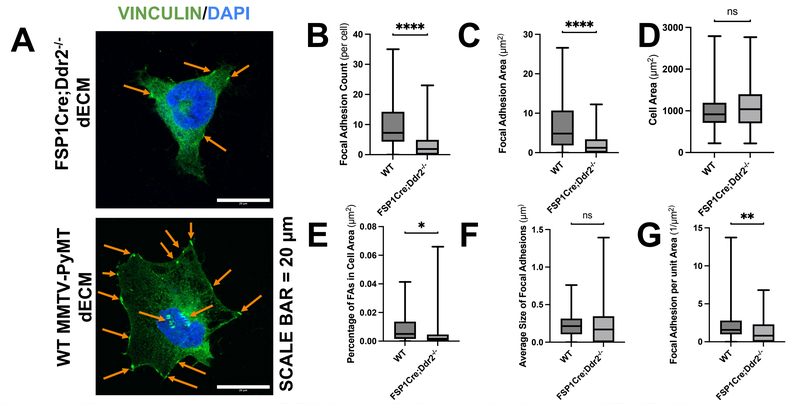


**Figure 4. Focal adhesions of BT549s on FSP1Cre;Ddr2$^{-/-}$ and WT dECM.** (A) Representative immunofluorescence images of focal adhesions on FSP1Cre;DDR2$^{-/-}$ dECM and WT dECM stained for vinculin (yellow arrows) and DAPI. (B) Number of focal adhesions per cell. (C) Area of focal adhesions on the cells. (D) Cell areas. (E) Percentage of total cell area occupied by focal adhesions. (F) Average size of each focal adhesion. (G) Number of focal adhesion per unit area of each cell. n=66-68 cells from two independent replicates; *p<.001,**p≈.001, ****p<.0001; t-Test with Welch's correction.

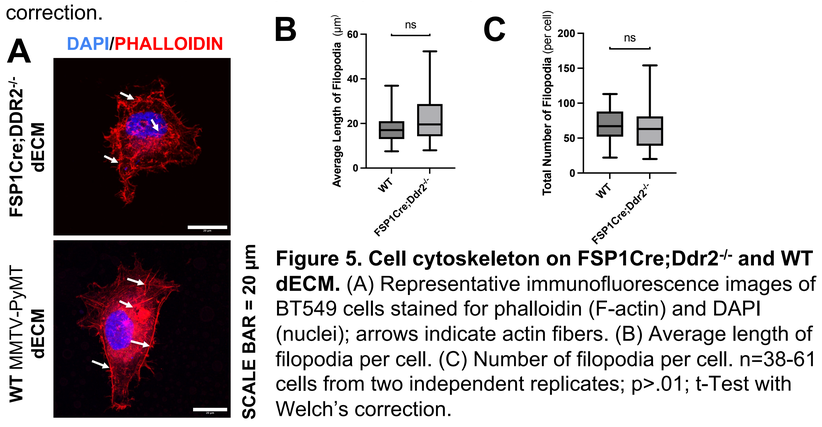


**Figure 5. Cell cytoskeleton on FSP1Cre;Ddr2$^{-/-}$ and WT dECM.** (A) Representative immunofluorescence images of BT549 cells stained for phalloidin (F-actin) and DAPI (nuclei); arrows indicate actin fibers. (B) Average length of filopodia per cell. (C) Number of filopodia per cell. n=38-61 cells from two independent replicates; p>.01; t-Test with Welch's correction.

## RESULTS & DISCUSSION

**Collectively migrating tumor cells form less focal adhesions on FSP1Cre;Ddr2$^{-/-}$ dECM.**

We observe less focal adhesions in the tumor organoids on the FSP1Cre;Ddr2$^{-/-}$ dECM than the wild-type MMTV-PyMT dECM (Fig. 6).

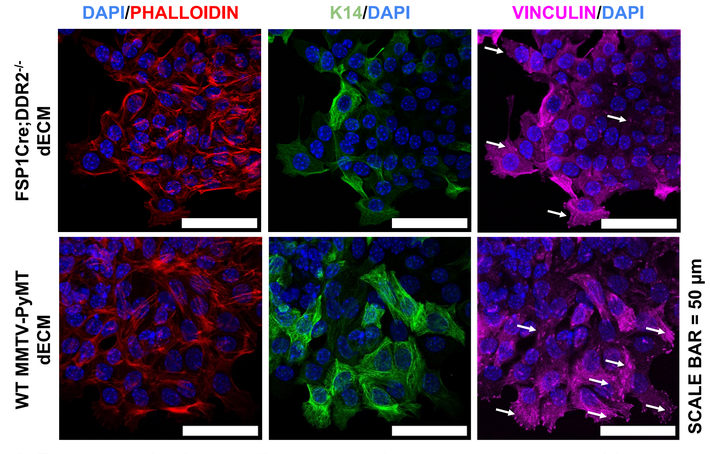


**Figure 6.** Representative immunofluorescence images of tumor organoids on FSP1Cre;Ddr2$^{-/-}$ and WT dECM with staining of actin (phalloidin), K14 (leader cell marker), vinculin (focal adhesions), and nuclei (DAPI). Arrows indicate focal adhesions.

## CONCLUSIONS

- BT549 cells form fewer focal adhesions on FSP1Cre;Ddr2$^{-/-}$ dECM compared to cell on wild-type MMTV-PyMT dECM. Similarly, we observe tumor organoids form fewer focal adhesions on FSP1Cre;Ddr2$^{-/-}$ dECM compared to cell on wild-type MMTV-PyMT dECM. Together, these findings suggest leader cells' ability to mechanically interact with the matrix is reduced when CAFs are modulated in the tumor microenvironment.
- We observe BT549 cells form less actin stress fibers on FSP1Cre;Ddr2$^{-/-}$ dECM compared to BT549 cells on wild-type MMTV-PyMT dECM, suggesting the cells are contracting on the FSP1Cre;Ddr2$^{-/-}$ dECM less than on the WT matrix.

## FUTURE DIRECTIONS

- We will quantify differences between focal adhesions at cell edges and centers on BT549s and tumor organoids using FIJI.
- We will investigate focal adhesion lifetime using live cell imaging with BT549s to see if changes in matrix affect cells' ability to maintain focal adhesions.
- We will quantify actin fiber length and orientation.
- We will evaluate tumor organoid behavior in a 3D FSP1Cre;Ddr2$^{-/-}$ dECM hydrogel.

## ACKNOWLEDGEMENTS

Thanks to NSF REU for their support and the Nanocharacterization Core for use of their facility. Thanks to the Heise and Puetzer labs for equipment use.

## REFERENCES

1. Polidoro, *World J Gastroentorol* 2020
2. Ping, Cancer Gene Ther. 2021
3. Bayer, *eLife* 2019
4. Link, *J. Vis. Exp*. 2017
5. Nulifar, *BMC systems biology 2013*