# Extracellular matrix stiffness regulates metabolic state in metastatic, but not quiescent, breast carcinoma cells

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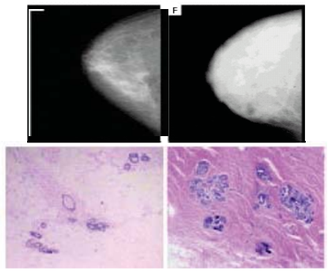
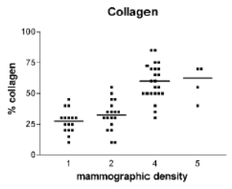
## Abstract

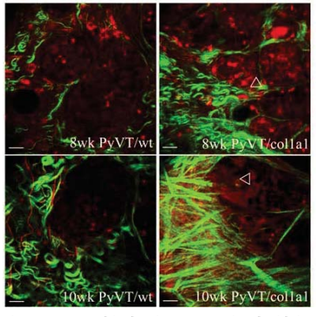
The strongest risk factor for developing breast cancer is age. However, this age related increase in risk is not the same for all women. Other risk factors can substantially add to the risk of developing breast cancer as a person ages. Increased breast density is associated with a 4-6 fold increased risk of developing breast cancer, and is associated with an increase in deposition of extracellular matrix (ECM) proteins, most abundantly collagen I. Collagen, like other ECM proteins, plays a structural role fundamental to tissue organization. High levels of collagen deposition correspond with a stiffer ECM, which is emerging as an important regulator of cell proliferation and tumor progression. Our previously reported microarray implicated changes in mammary epithelial cell metabolism in response to increased matrix stiffness, consistent with the expanding role of the ECM in tumor cell signaling. Here we report that increased matrix stiffness regulates metabolic enzymes including pyruvate dehydrogenase kinase 1, a key regulator between lactic acid production and pyruvate entry into the mitochondria, in highly metastatic 4T1 breast carcinoma cells. Interestingly, we do not observe this same metabolic regulation in quiescent (dormant) 4T07 tumor cells of the same lineage. While these mRNA and protein changes suggest an increase in aerobic metabolism in metastatic cells, we surprisingly find that highly metastatic cells have increased oxygen consumption in response to a compliant extracellular matrix. Moreover, we find an uncoupling of glycolysis and the tricarboxylic acid (TCA) cycle in 4T1 cells in response to a stiff ECM but not a compliant ECM. Addition of glutamine to minimal media causes an increase in oxygen consumption in 4T1 cells in a stiff but not compliant matrix. These changes in metabolism are not present in the quiescent 4T07 cells. Moreover, glutamine utilization in the TCA cycle is increased in metastatic cells in a stiff matrix. The quiescent 4T07 cells do not appear to alter their glutamine flux to the same degree in response to changes in ECM stiffness. Thus, we find that alterations in collagen stiffness cause metabolic shifts between oxidative phosphorylation and aerobic glycolysis in highly metastatic cells, but not in quiescent cells. These findings identify stiffness of the ECM as an important regulator of metabolic state, and further identify quiescence as a dominant trait that is not overcome by ECM stiffness.

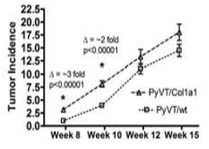
## Increased mammographic density is associated with increased collagen in breast

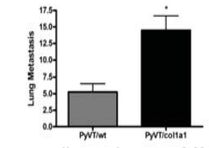





## Increased breast density is associated with increased tumor development and aggressiveness




- Increases in mammographic density are associated with increases in collagen I deposition [1][2]
- A mouse model of increased breast density showed an increase in tumorigenesis and metastasis to the lung compared to animals with normal extracellular collagen density [3]

**References:**

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2. Guo Y-P, Martin LJ, Hanna W, Banerjee D, Miller N, Fishell E, Khokha R, Boyd NF. Growth factors and stromal matrix proteins associated with mammographic densities. Cancer Epidemiology Biomarkers & Prevention. 2001;10(3):243-8.
3. Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, White JG, Keely PJ. Collagen density promotes mammary tumor initiation and progression. BMC medicine. 2008;6(1):11.

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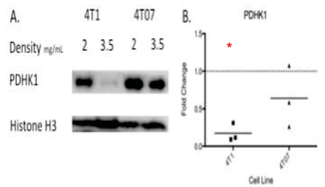
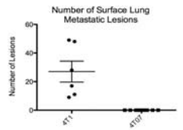
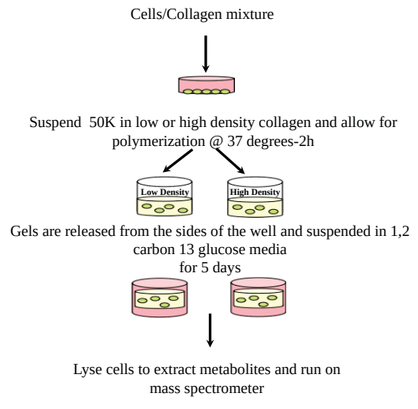
## mRNA regulation of metabolic genes in response to a dense collagen matrix

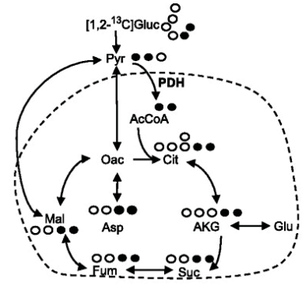


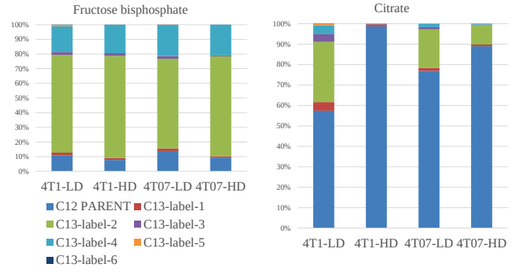

- Expression changes in response to a high density collagen ECM
- Green = enzyme downregulation Red = enzyme upregulation
- A high density collagen matrix caused an increase in mRNA transcripts of genes associated with the tricarboxylic acid (TCA) cycle, the electron transport chain and the pentose phosphate pathway
- Glycolytic genes were downregulated in a high density collagen matrix.
- Confirmed at protein level for key enzymes in highly metastatic (4T1) but not quiescent (4T07) breast carcinoma cells
- 4T1 vs 4T07 cells – taken from same spontaneous mouse mammary tumor; isolated based on lung metastatic potential
  - 4T1 traffics to lung and forms surface mets, 4T07 traffics to lung but arrests before proliferating

## Steady State Metabolite Labelling: How it works




## Labeled 1,2-13C glucose steady state metabolite labeling

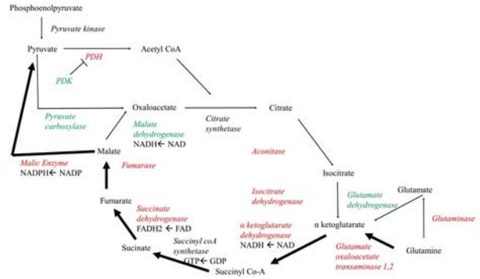






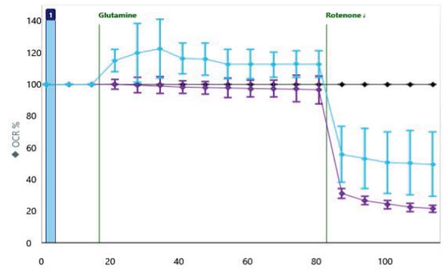
- Similar labeling patterns of carbon atoms in fructose bisphosphate and lactate were seen in 4T1 and 4T07 cells in response to changes in extracellular collagen density
- 4T1 cells in a high density collagen matrix showed almost no labeling of citrate and other TCA cycle intermediates, suggesting a decreased flux between glycolysis and the TCA cycle in metastatic cells in response to a dense collagen matrix.
- This same degree of change in glycolysis and TCA flux was not seen in dormant 4T07 cells.

## Could change in metabolic enzyme expression be due to glutamine switch?




- Upregulation of genes associated with oxidation of glutamine to pyruvate in response to a dense collagen matrix in our microarray
- Others have found a switch to glutamine metabolism is necessary for tumor growth and progression

## Changes in oxygen consumption in response to addition of glutamine




- Addition of glutamine causes an increase in oxygen consumption in 4T1 cells in a high density collagen matrix
- Graph shows the percent change in oxygen consumption from baseline after addition of glutamine (first green line)
- Rotenone and Antimycin was injected at second green line as an internal control to ensure 3D spheroids remained functional throughout SeaHorse experiment

## Discussion and Future Directions:

- Metastatic 4T1 cells undergo an uncoupling of glycolysis and the TCA cycle in response to a dense collagen ECM
  - Possibly due to a glutamine switch in metabolism
- Quiescent 4T07 cells do not undergo the same uncoupling
  - Response to glutamine remains unknown
- Increase in TCA cycle genes on our initial microarray may be due to enhanced glutaminolysis
- Perform labeled glutamine flux analysis
- Mechanism for glutamine metabolic switch is currently unknown. Others have shown increased ERK signaling leads to increased glutamine metabolism
  - We have seen increased ERK activity in 4T1 HD cells compared to LD but not in 4T07 cells
  - Glutaminolysis enzymes decrease in HD collagen matrix when treated with ERK inhibitor (not shown) suggesting role for ERK in this glutamine switch