

Aging and pulmonary fibrosis regulates the quiescent state of metastatic mammary carcinoma cells

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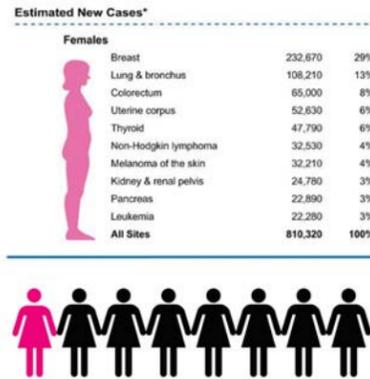
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Abstract

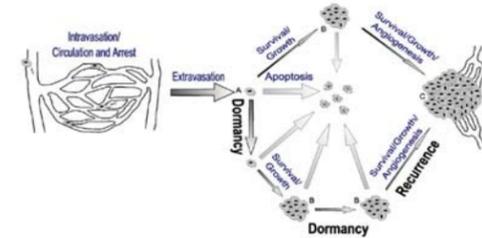
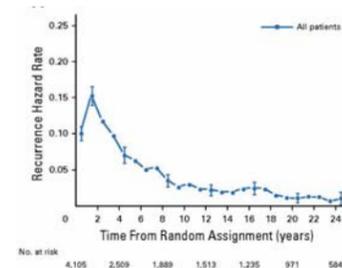
Metastatic disease accounts for the majority of cancer related deaths. However, the process is relatively inefficient, with only a few cells successfully leaving a primary tumor to colonize distant sites throughout the body. Moreover, some metastatic tumor cells enter into a state of dormancy at the metastatic site, remaining as small, undetectable lesions until some unknown cue triggers these cells to start proliferating and cause clinically detectable metastatic disease. These latent metastatic lesions have occurred sometimes as long as 20 years after conclusive treatment for the primary tumor, suggesting that the metastatic cells can remain dormant within the body for exceptionally long periods of time. In this study, we aimed to determine what cues occurring during the aging process might trigger dormant cells to become proliferative at the metastatic site. By utilizing a mammary carcinoma cell line (4T07) that is known to traffic to but remain dormant at the mouse lung, we evaluated changes in the metastatic potential of these cells in response to changes in the local extracellular matrix of aged animals. We found that when these dormant 4T07 cells were placed in aged animals, the 4T07 cells exited dormancy and formed detectable metastatic lesions at the mouse lung, suggesting that something about the aged mouse lung could overcome the inherent dormancy of these cells. We further narrowed down what was causing the 4T07 cells to exit dormancy by showing that by increasing lung fibrosis in young animals, we could recapitulate our finding from the aged animals of 4T07 cells exiting dormancy to form detectable lung metastases. By performing whole lung proteomics on fibrotic mouse lungs versus control lungs, we identified an upregulation of the small leucine rich proteoglycan lumican, a protein important in the structural organization of collagen, in our fibrotic lungs. Immunohistochemical staining of lung samples confirmed the increase of lumican in fibrotic lungs. Lumican has been broadly implicated in the progression and spread of cancer and future experiments will elucidate whether it is necessary for the change in tumor cell dormancy we observe in fibrotic lungs. Our findings shed light on the role of aging, specifically changes in lung fibrosis on the progression and metastatic potential of tumor cells. It also provides important insights into the cues which may trigger dormant cells to begin proliferating and form clinically detectable metastatic lesions.

Breast Cancer Significance

- Breast Cancer is the most commonly diagnosed cancer among women in the United States
- About 1 in 8 women in the United States will be diagnosed with invasive breast cancer in their lifetime



Breast Cancer Dormancy and Recurrence



- The risk of recurrence is greatest 1-2 years after surgery and definitive therapy for breast cancer
- The risk of recurrence does not return to zero after definitive therapy for breast cancer
- Some patients have developed recurrent disease as long as 30 years after therapy

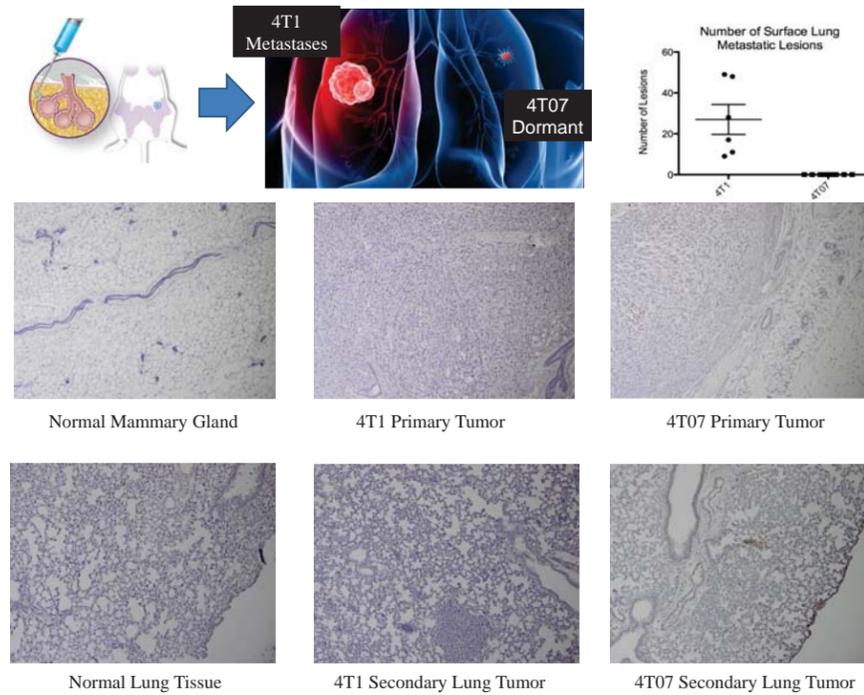
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Acknowledgements:

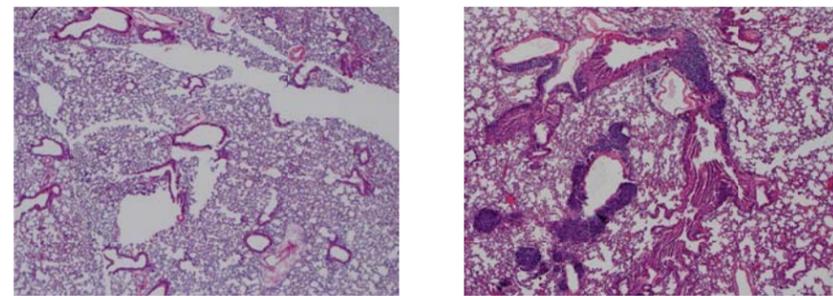
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Mouse Models of Breast Cancer and Dormancy



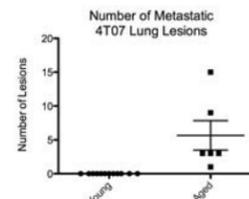
Aged mice cause 4T07 cells to exit dormancy and form metastatic lesions

- Average Mouse Lifespan is about 29 months
- Injected 4T07 cells into young (6 month) and aged (26 month) mice
- 4T07 cells in aged mice trafficked to lung and formed large metastatic lesions



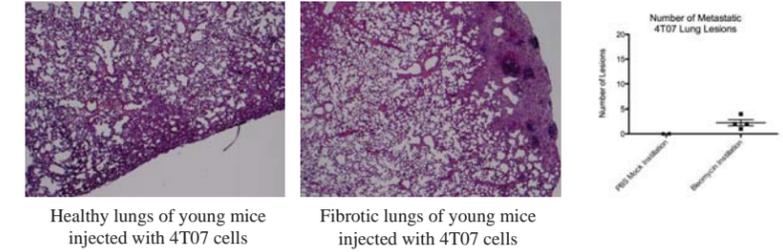
Lungs of young mice injected with 4T07 cells

Lungs of aged mice injected with 4T07 cells



A fibrotic lung microenvironment causes 4T07 cells to exit dormancy and form metastatic lesions

- Aged lungs are known to have increased fibrosis
- By inducing lung fibrosis in young animals, we found that 4T07 cells would exit dormancy, similar to when these cells were placed in an aged animal



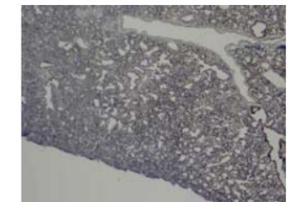
Healthy lungs of young mice injected with 4T07 cells

Fibrotic lungs of young mice injected with 4T07 cells

Fibrotic Lungs show an increase in Lumican expression

- We performed whole lung proteomics to identify differential expression of proteins in young lungs that were either fibrotic or healthy
- From this screen, we identified the small leucine rich proteoglycan lumican
- We confirmed this increase in lumican on our tissue sections via staining
- Lumican is associated with progression and spread of breast cancer

Identified Proteins	B/C	p value
Sarcoendoplasmic reticulum chaperone	0.000	0.0001
Ras-related protein R-Ras	#DIV/0!	0.0002
Protein kinase, cAMP dependent regulatory, type II alpha	1.721	0.004
Cytochrome c oxidase subunit 2	2.463	0.006
Lamin-B1	0.721	0.006
Isiorm 2 of Cell surface glycoprotein MUC18	#DIV/0!	0.007
ADP/ATP translocase 2	0.561	0.011
45S ribosomal protein S4, X isoform	#DIV/0!	0.012
Nucleolin	#DIV/0!	0.015
Isiorm 2 of Secin	0.205	0.019
Alpha actinin 1a	1.534	0.019
Chaperonin	1.768	0.021
Anaxin A3	0.603	0.028
10 kDa heat shock protein, mitochondrial	#DIV/0!	0.029
Stress-70 protein, mitochondrial	#DIV/0!	0.032
Carbonic dehydrase (NADPH) 2	0.715	0.033
Dipeptidase 1	0.362	0.034
Lumican	2.796	0.034
45S ribosomal protein S12	2.880	0.035
Vicilin	0.769	0.035
Alpha-globin	0.447	0.040
Glutathione S-transferase P 1	0.591	0.044
Isiorm 2 of NADH-cytochrome b5 reductase 3	1.714	0.046
Carbonic anhydrase 2	0.355	0.048
Talin-1	0.739	0.054



Healthy lungs of young mice injected with 4T07 cells show lower amounts of Lumican expression



Fibrotic lungs of young mice injected with 4T07 cells show higher amounts of Lumican expression throughout the entire lung

Tumor	Relative abundance of lumican in tumor as compared to benign tissue	Lumican expression as correlated to disease stage	Reference
Breast cancer	Fibroblast-like cells within stroma	↑ lumican expression was associated with higher tumor grade	Leygue et al., 1996 and Leygue et al., 2000
Tumor tissue	↑	↑ lumican expression in infiltrating ductal carcinoma was associated with higher tumor grade	Somiari et al. (2003)
Tumor tissue	↓	↓ expression in advanced disease as compared to early disease	Eshchenko et al (2007)
Tumor tissue	↓	↓ expression of lumican is associated with poor outcome in node negative breast cancer	Paris et al (2013) Troup et al (2003)

Discussion and Future Directions:

- Our findings shed light on the role of aging, specifically changes in lung fibrosis on the progression and metastatic potential of tumor cells.
- We provide important insights into the cues which may trigger dormant cells to begin proliferating and form clinically detectable metastatic lesions.
- Future experiments will elucidate whether Lumican is necessary for the change in tumor cell dormancy we observe in fibrotic lungs