The paradoxical role of Runx1 in collective invasion and metastasis

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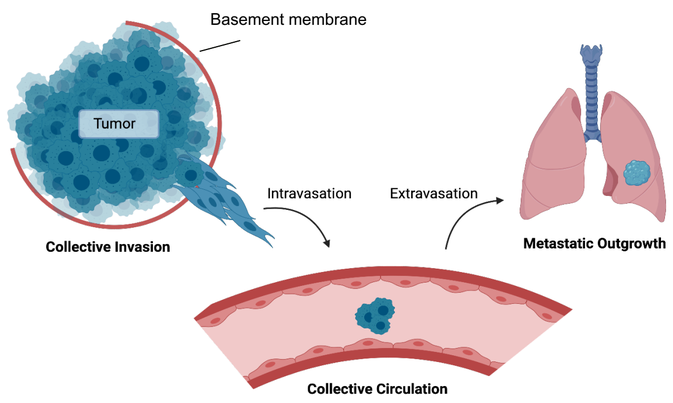
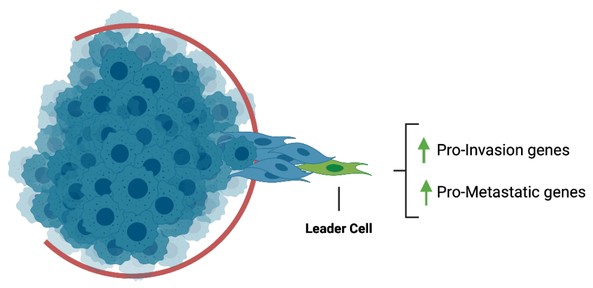
Introduction

Collective invasion and dissemination are commonly observed in breast cancer. [1,2]



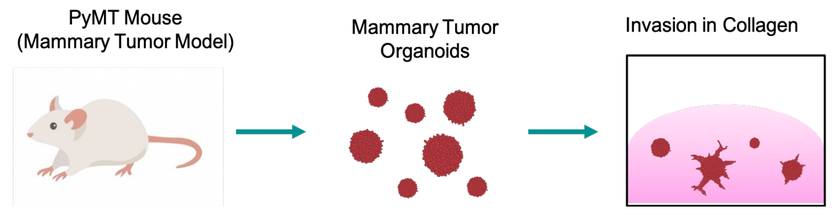
The cells leading collective invasion, called "leader cells" upregulate transcription of several genes that contribute to invasion, as well as metastatic seeding and outgrowth. [3,4]



It is still unclear how this transcriptional state arises, and why it occurs in only a subset of tumor cells.

Methods

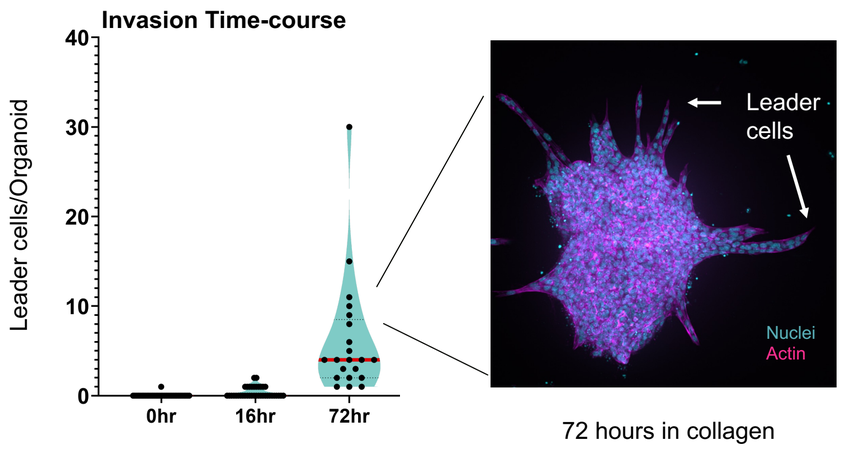
We model collective invasion by embedding PyMT mammary tumor organoids into Collagen I. [5]



Objective: Define the transcriptional state of breast cancer leader cells and use it to identify transcriptional regulators of leader cells.

Results

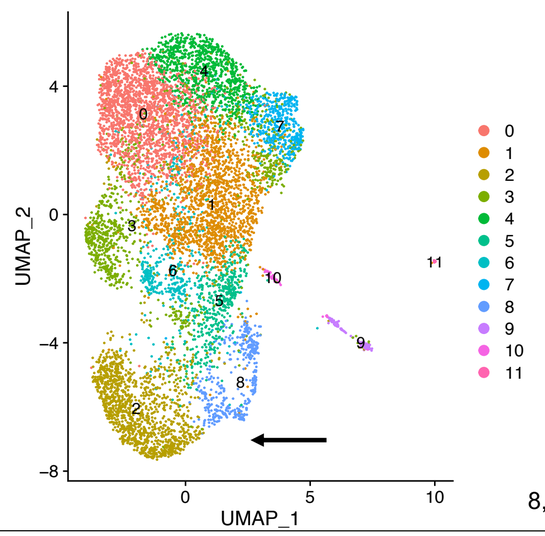
Leader cells become more abundant over time in 3D collagen matrices.



72 hours in collagen

Using cells from each time point, we performed sc-RNAseq to uncover the transcriptional state of organoid cells during invasion. We found that cluster 2 has the highest expression of leader cell genes and its population correlates highly with leader cell counts from each time point.

Clusters called by Seurat[6]



Correlation with invasion

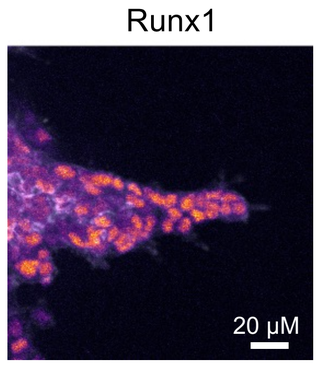
Cluster	R-Value
0	0.4286
1	-0.8286
2	0.9429
3	-0.4857
4	0.2571
5	-0.7143
6	-0.6
7	0.6571
8	-0.2571
9	0.08571
10	0.3143
11	-0.03036

8,930 cells total, 2 biological replicates

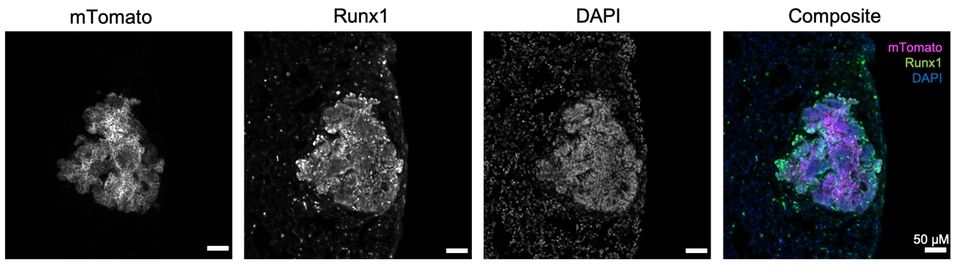
We stained invaded organoids for transcription factors (TFs) upregulated in cluster 2 and found that Runx1 is specifically high in leader cells.

Cluster 2		
TF	Fold Change	Result
Atf3	2.03163876	High in Leaders
Trp63	1.94057716	High in Leaders
Runx1	1.93255779	High in Leaders
Id3	1.88399069	High in Leaders
Tsc22d1	2.70250912	Not detected
Klf4	1.81528955	Not detected
Jun	2.41703818	Not checked
Fos	2.34205486	Not checked
Sox4	1.97274809	Not checked
Junb	1.77641953	Not checked
Egr1	1.73433088	Not checked

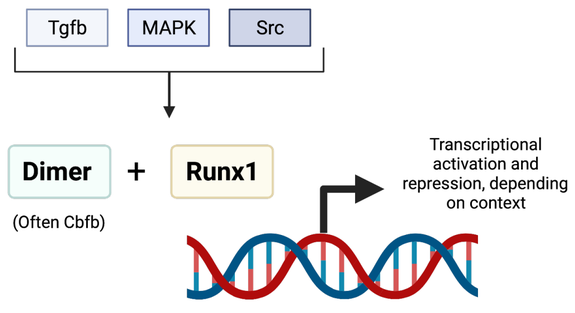
Runx1



Runx1 is also upregulated in mTomato-PyMT lung metastases.

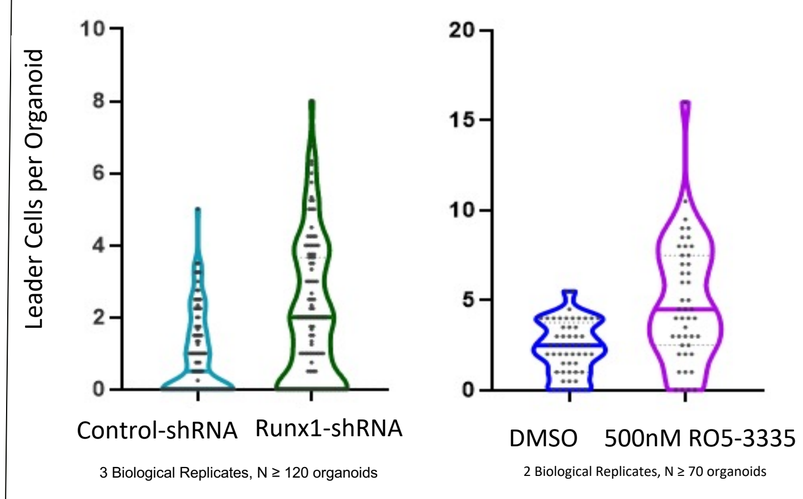


A little about Runx1:

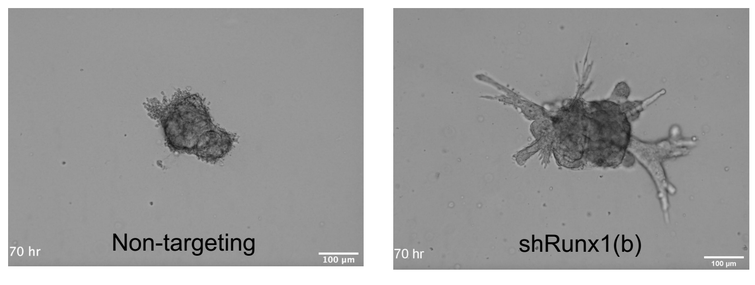


- **Important for:** Hematopoiesis, Bone development, Neuronal development, Mammary development. [7]
- **Breast cancer survival:** Loss of function mutations and lower expression levels correlate with worse outcomes in patients. [7]
- **Inhibited by** the small molecule inhibitor RO5-3335. [8]

Unexpectedly, inhibition of Runx1 with shRNA interference or Ro5-3335 treatment led to an increase in invasion.

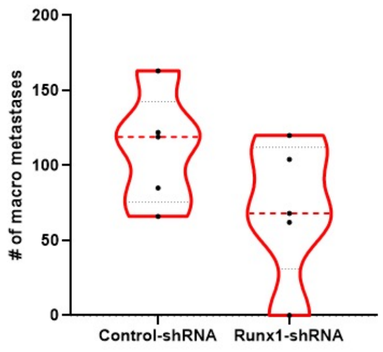


Representative time-lapse endpoint images

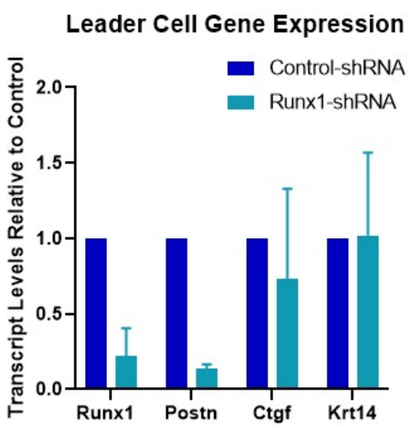


To assess the role of Runx1 in metastatic seeding, we injected knockdown cells into the tail veins of mice and collected the lungs 3 weeks later to count metastases.

Interestingly, Runx1 appears to support metastatic outgrowth in the lungs. However, this assay skips the need for invasion.



We used qPCR for top leader cell genes to determine that Runx1 does not control transcription of all leader cell genes, but may regulate a subset.



Conclusions

- Runx1 is upregulated in PyMT leader cells in a 3D culture model.
- Runx1 is upregulated in PyMT lung metastases in-vivo.
- Contrary to what we hypothesized, Runx1 may restrict leader cell formation and function.
- Paradoxically, Runx1 also appears to support metastatic seeding and outgrowth.
- While Runx1 is not the sole transcription factor that regulates leader cells, it appears to be a contributor.

Future Directions

- Determine whether Runx1 regulates invasion in-vivo. Repeat tail vein experiments

- Use RNA-seq to find out which genes Runx1 is activating and suppressing in a 3D collagen environment.
- Expand Runx1 invasion studies to human samples.
- Determine how reliant the Runx1 invasion phenotype is on Postn.

Citations

1. Aceto N. et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell. Aug 28;158(5):1110-1122. doi: 10.1016/j.cell.2014.07.013. (2014)
2. Khalil, A. A. et al. Collective invasion in ductal and lobular breast cancer associates with distant metastasis. Clin. Exp. Metastasis 34, 421–429 (2017)
3. Cheung, K. J., Gabrielson, E., Werb, Z. & Ewald, A. J. Collective Invasion in Breast Cancer Requires a Conserved Basal Epithelial Program. Cell 155, 1639–1651 (2013).
4. Mayor R, Etienne-Manneville S. The front and rear of collective cell migration. Nat Rev Mol Cell Biol. Feb;17(2):97-109. doi: 10.1038/nrm.2015.14. (2016)
5. Nguyen-Ngoc KV. et al ECM microenvironment regulates collective migration and local dissemination in normal and malignant mammary epithelium. Proc Natl Acad Sci U S A. Sep 25;109(39):E2595-604. doi: 10.1073/pnas.1212834109. (2012)
6. Stuart T. et al. Comprehensive Integration of Single-Cell Data. Cell. 2019 Jun 13;177(7):1888-1902.e21. doi: 10.1016/j.cell.2019.05.031. (2019)
7. Hong D. et al. RUNX1-dependent mechanisms in biological control and dysregulation in cancer. J Cell Physiol. 2019 Jun;234(6):8597-8609. doi: 10.1002/jcp.27841. (2019)
8. Cunningham L. et al. Identification of benzodiazepine Ro5-3335 as an inhibitor of CBF leukemia through quantitative high throughput screen against RUNX1-CBFβ interaction. Proc Natl Acad Sci U S A. 2012 Sep 4;109(36):14592-7. doi: 10.1073/pnas.1200037109. (2012)

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