Elucidating the Niche Microenvironments of Dormant and Metastatic Breast Cancers

Nafiza Meher, Sreeharsha Gurrapu, Ph.D. , Filippo Giancotti M.D. Ph.D.

Giancotti Laboratory, The University of Texas MD Anderson Cancer Center, Houston, TX

Background

- Metastatic relapse can occur in dormant tumors. This has been observed in many different cancers, including Breast cancer.
- Such metastasis can occur due to rare subclones, a.k.a. cells from the primary tumors that diverge due to differences in mutations [1,2,3].
- New micro-metastases are more prone to attacks from the immune system until they develop the ability to evade the immune system and re-establish an immunosuppressed microenvironment for cancer growth.
- Possible complex signals control the reactivation of dormant cells triggering subclones and thus micro-metastases in niches [4].
- Possible analogous system between that and the development of stem cells through regulation by immune cell niches [5, 6].

Methods

- **To Identify the niche cell composition of dormant and metastatic tumors though genetic labeling**

1. Dormant breast cancer cell line 4TO7 and metastatic 4T1 cells were modified to express eGFP and soluble mCherry labelling dye so that the metastatic niches can be labeled [7].
2. Also modified to express reporter gene luciferase, in order to study metastatic reactivation in vivo.
3. Breast tumor cells are injected into the tail vein of syngeneic mice. The cells seed into the lungs of the female BALB/c mice.
5. Tail vein injected 4TO7 cells enter a dormant stage and 4T1 cells undergo metastatic reactivation. This allows us to examine the dormant niches (from day 3), early (≥ 1 week) and late reactivated niches (≥ 2 weeks), and macro-metastases (≥ 3 weeks).
6. The target organs of the mice are collected and tumors and niche cells are isolated by fluorescence-activated cell sorting. eGFP positive and mCherry positive cells are tumor cells, and only mCherry positive cells are the niche cells.
7. The cells undergo scRNA-sequencing, bioinformatics and single-cell cluster analyses.

Hypothesis

We hypothesize that metastasis-initiating cells reside within specific niches that support their survival and self-renewal capacity. Moreover, the dormant and metastatic cells might have a distinct niche and stromal cell subsets that regulate metastasis.

Results

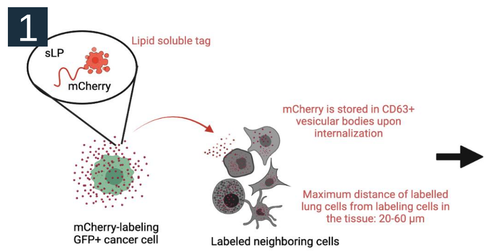

Figure 1. Cells are labeled with eGFP and mCherry. eGFP positive and mCherry positive cells are tumor cells. Specifics of the mCherry labeling is shown in the figure.

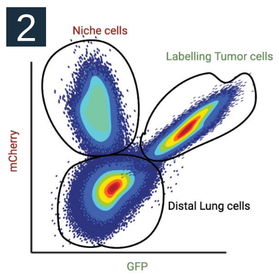

Figure 2. eGFP positive and mCherry positive cells are tumor cells. Niche cells are mCherry positive. Distal lung cells are eGFP and mCherry negative.

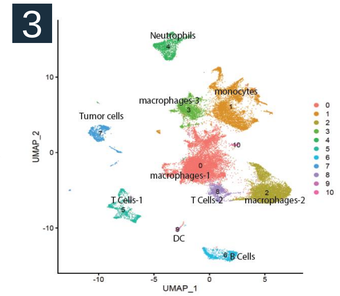

Figure 3. Single cell sorting of tumor cells and niche cells showed the following distribution.

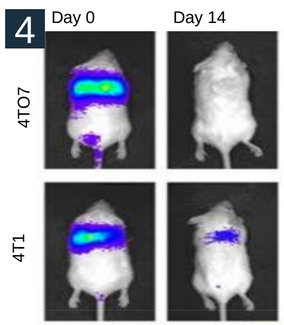

Figure 4. The figure shows images of representative models of mice with 4T07 and 4T1 cancer cells on Day 0 and Day 14. Express reporter gene luciferase shows metastatic reactivation.

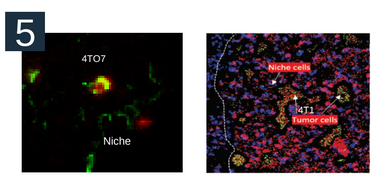

Figure 5. 4T07 micro-metastasis is shown. The 4T07 cancer cells and the niche is labeled. 4T1 micro-metastasis is shown. The 4T1 cancer cells and the niche is labeled.

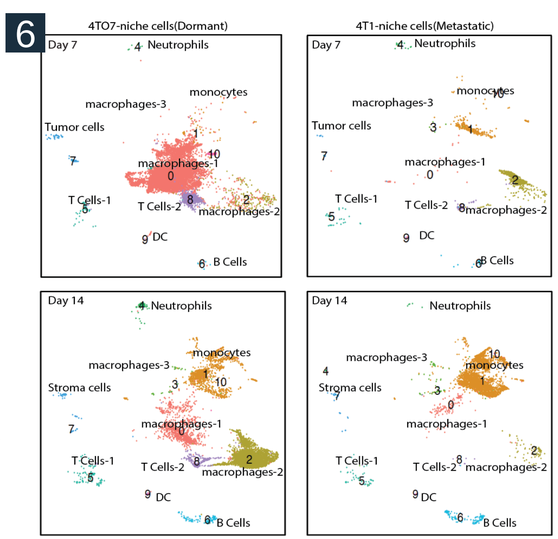

Figure 6. Our preliminary data show that dormant and reactivated niches are mostly immune subpopulations. Dormant niches show a large presence of macrophages, B cells, and T cells. In contrast, the reactivated metastatic niches show an increased number of monocytes but a decrease in macrophages, B cells, and T cells.

Discussion

- Examining the niches of the cancer cells helps us identify the differences in dormant cells and metastatic cells surrounding growth conditions.
- Our data suggest the possibility of metastatic cells evading the immune system by building a niche that activates specific signaling pathways that allow for immune evasion and immunosuppression.

Future Directions

- Identify the niches specifics and conduct further research into these pathways and mechanisms
- Decipher how to target the reactivation of metastasis.
- Possible lead to the development of novel biomarkers and therapeutics to treat cancer.

References

1) Ding, L., et al., *Genome remodelling in a basal-like breast cancer metastasis and xenograft.* Nature, 2010. **464**(7291): p. 999-1005.
2) Gerlinger, M., et al., *Intratumor heterogeneity and branched evolution revealed by multiregion sequencing.* N Engl J Med, 2012. **366**(10): p. 883-892.
3) Wu, X., et al., *Clonal selection drives genetic divergence of metastatic medulloblastoma.* Nature, 2012. **482**(7386): p. 529-33.
4) Giancotti, F.G., Mechanisms governing metastatic dormancy and reactivation. Cell, 2013. 155(4): p. 750-64.
5) Su, W., et al., *The Polycomb Repressor Complex 1 Drives Double-Negative Prostate Cancer Metastasis by Coordinating Stemness and Immune Suppression.* Cancer Cell, 2019. **36**(2): p. 139-155.e10.
6) Naik, S., et al., *Inflammatory memory sensitizes skin epithelial stem cells to tissue damage.* Nature, 2017. **550**(7677): p. 475-480.
7) Ombrato, L., et al., *Metastatic-niche labelling reveals parenchymal cells with stem features.* Nature, 2019. **572**(7771): p. 603-608.

Acknowledgements

This presentation is supported by the CPRIT, NIH and MD Anderson Cancer Center, through the “The Future of Cancer Research: Training Program for Basic and Translational Scientists” grant, awarded for the CPRIT-CURE Summer Undergraduate Program. Additional thanks to the Giancotti Laboratory, Dr. Giancotti, and Dr. Gurrapu for allowing me to be a part of their lab and their research this summer.