



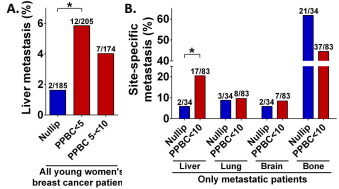
RNA-seq analysis of murine liver to identify breast cancer metastatic potential during liver involution

Michelle K. Ozaki, Alexandra Q. Bartlett, Zheng Xia, & Pepper Schedin
Department of Cell, Developmental, and Cancer Biology, Oregon Health and Science University



Background

Postpartum breast cancer cases have increased risk of liver metastasis:



Postpartum breast cancer (PPBC) is defined as breast cancer diagnosed within 10 years of last pregnancy. PPBC patients have an increased risk of developing liver metastases compared to never pregnant (nullip) patients (Fig 1).

Fig 1. A) Frequency of liver metastasis in nulliparous and PPBC patients from a University of Colorado young women's cohort (n=564; p=0.03). B) Frequency of metastasis to the liver, lung, brain, and bone in nulliparous and PPBC patients that had metastases.

Post-wean period is a key window for tumor advantage:

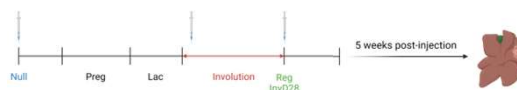


Fig 2. Mice were injected via the portal vein with mammary D2A1 murine tumor cells at nullip, involution, or regressed states. Tumors were examined and quantified 5 weeks post-injection.

Only mice injected during involution had increased tumor burden identifying the window of involution as an opportunistic period of tumor advantage.

The liver undergoes tissue remodeling in response to a reproductive cycle

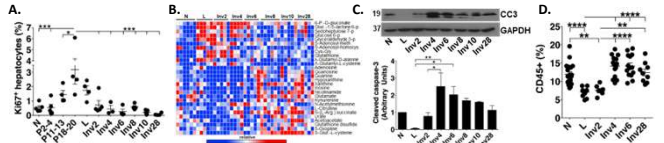
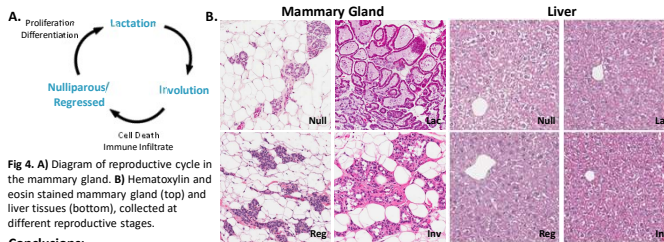


Fig 3. A) Quantification of Ki67 positive hepatocytes, B) metabolic profiling, C) apoptosis as measured by cleaved caspase 3 expression by western blot, and D) percent CD45+ cells as measured by flow cytometry across a full reproductive cycle.



Conclusions: Mammary gland and the liver are a functional unit, with the liver showing molecular signs of proliferation, differentiation, and involution that mirror the reproductive cycle in the mammary gland (Fig 3). In the liver, the reproductive cycle, including involution, is much more subtle at the histological level than in the mammary gland (Fig 4). To date, what we know about liver involution has been guided by findings from the mammary gland, but because of these tissue differences further molecular analyses are needed.

Research Questions & Methods

- 1. What can we learn about liver involution from an unbiased liver RNA-seq approach?
- 2. Can we find further evidence for why the involution supports a metastatic niche?

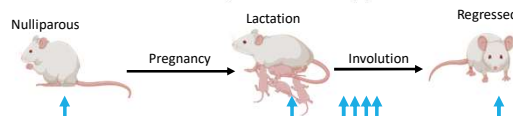


Fig 5. Breeding scheme for liver RNA isolation. Mice are bred, weaned after ~10 days of lactation, and are at a regressed state at 4 weeks post-wean. RNA isolated at time points depicted by blue arrows. Nullip (Null) (n=4), Lactation (Lac) (n=3), involution day 2 (ID2) (n=3), ID4 (n=4), ID6 (n=6), ID8 (n=3), Regressed (Reg) (n=4).

Results

Liver RNA-seq data show evidence of a classic reproductive cycle:

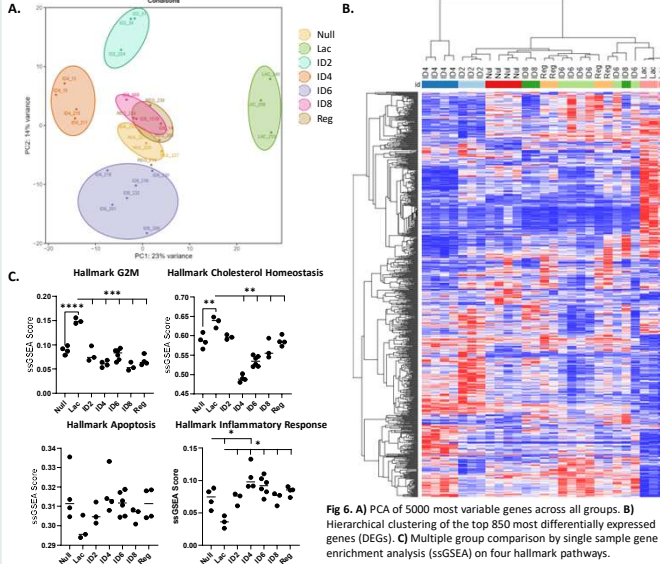
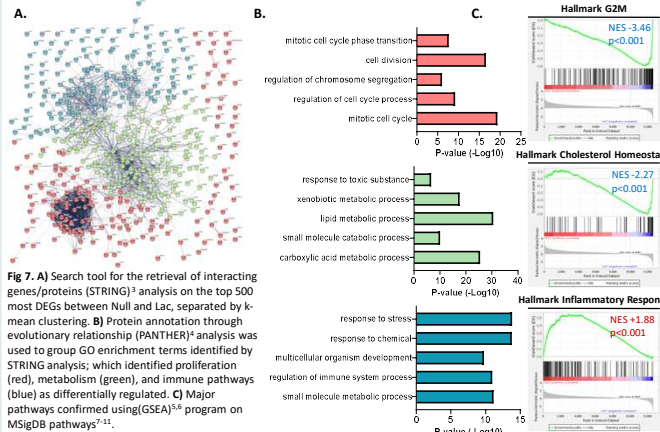


Fig 6. A) PCA of 5000 most variable genes across all groups. B) Hierarchical clustering of the top 850 most differentially expressed genes (DEGs). C) Multiple group comparison by single sample gene set enrichment analysis (ssGSEA) on four hallmark pathways.

Two group comparisons show proliferation and metabolism are increased during lactation:



Two group comparisons show apoptotic cell death enriched during involution

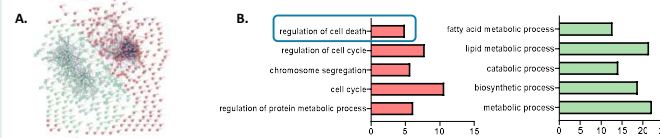
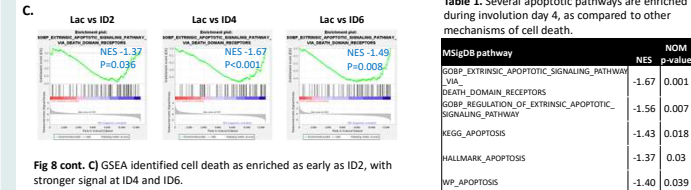


Fig 8. A) The top 500 DEGs between Lac and ID2 were analyzed by STRING analysis, and separated into clusters. B) PANTHER analysis on these two clusters identified cell death as an enriched pathway, along with proliferation and metabolism signatures.

Results Continued



Evidence for immune cell infiltrate during involution:

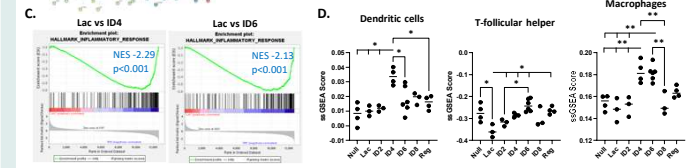
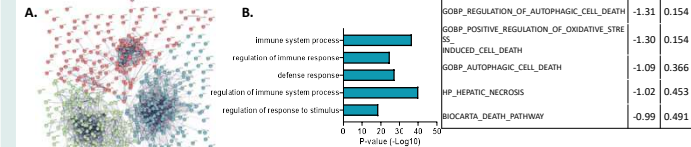


Fig 9. A) STRING and B) PANTHER analysis show a main cluster identified between Lac and ID4 is immune related (blue). C) Two group comparisons of ID4 or ID6 to lactation show increased enrichment of immune response genes at ID4 and ID6 by GSEA. D) ssGSEA was used to identify how different populations of immune cells (dendritic cells, T-follicular helper cells, and macrophages) are enriched during involution, with evidence for immune resolution by ID8.

Gene signatures associated with poor outcomes in breast cancer are found in the normal involuting liver:

- Previous studies have shown:
 - Mammary gland involution gene signatures associate with poor breast cancer outcomes
 - Immune cells, especially myeloid derived cells, associate with the liver metastatic niche
 - Extracellular matrix (ECM) and ability to remodel ECM promotes tumor growth

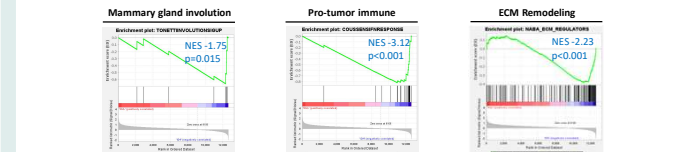


Fig 10. Pro-tumor microenvironment signatures found in the involution liver include: human mammary gland involution, rodent pro-tumor immune, and multispecies ECM remodeling. Comparisons by GSEA are between nulliparous and ID4 livers.

Conclusion

The involuting liver has features of the involuting mammary gland, but has distinct differences that may provide further insight into the liver metastatic niche. Two areas of interest for further investigation are the role of immune suppressive macrophages and ECM remodeling.

Acknowledgements & References

I would like to thank Dr. Pepper Schedin for her mentorship and guidance, Dr. Zheng Xia for his work and assistance on the computational and bioinformatics work. I'd like to acknowledge Dr. Nathan Pennock and Dr. Alexandra Quakenbush Bartlett, who completed the sequencing, and provided mentorship throughout this project. I would also like to thank the NSF GRFP and the ARCS Oregon Foundation for financially supporting my graduate schoolwork. And finally, I'd like to thank the rest of the Schedin lab for their continuous support.

References: 1) Goddard, E.T. et al. Cancer Discov. 2017. 2) Goddard, E.T. et al. J. Vis. Exp. 2016. 3) Snel, B., et al. Nucleic Acids Res. 2000. 4) Mi, H., et al. Nucleic Acids Res. 2021. 5) Subramanian, A., et al. Proc. Natl. Acad. Sci. 2005. 6) Moorthi, V.K., et al. Nat. Genet. 2003. 7) Liberson, A., et al. Cell Syst. 2015. 8) Fahrenholt, A., et al. Nucleic Acids Res. 2018. Martens, M., et al. Nat. Genet. 2000. 9) Martens, M., et al. Nucleic Acids Res. 2021. 10) Ashburner, M., et al. Nat. Genet. 2000. 11) The Gene Ontology Consortium, et al. 2021. Axtallos, S., et al. Cancer Prev. Phila. 2010. DeNardo, D.G., et al. Cancer Discov. 2011. Shao, X., et al. Nucleic Acids Res. 2020.

Unpublished data: Please do not share/post