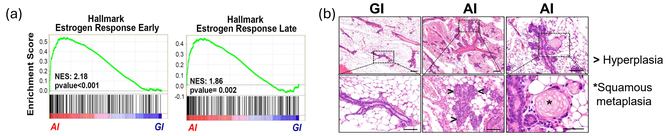
Single Cell Proteomic Phenotyping Reveals Mitigation of Abrupt Involution-Induced Alteration in Mammary Epithelium Upon Tamoxifen Treatment

[1]Sarmila Majumder PhD, [5]G. Kenneth Gray PhD, [1]Neelam Shinde MS, [1]Resham Mawalkar BS, [2]Sanjay Mishra PhD, [1]Allen Zhang MD, [1]Mustafa Basree DO, [1]Kate Ormison PhD, [3]Xiaoli Zhang PhD, [2]Ramesh Ganju PhD, [4]Gina M Sizemore PhD, [1]Bhuvaneswari Ramaswamy MD MRCP and [1]Daniel G Stover MD. [1]Dept. of Internal Medicine, [2]Dept. of Pathology, [3]Dept. of Statistics, [4]Dept. of Radiation Oncology, College of Medicine, The Ohio State University Comprehensive Cancer Center. [5] Dept. Of Cell Biology, Harvard Medical School.

THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER

INTRODUCTION

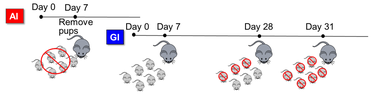
Epidemiological studies link lack of or short-term breast feeding to increased risk of breast cancer (BC), including triple negative breast cancer (TNBC)[1]. However, few models allow interrogation of the underlying biology. To decipher this link, we developed a model of short-term breastfeeding leading to **abrupt involution (AI)** versus long-term BF leading to **gradual involution (GI)** in mice. In our prior work, we showed that AI led to (a) significant increase in estrogen signaling and (b) precancerous changes (hyperplasia, squamous metaplasia) in mammary glands[2]. Further, AI glands are characterized by a notable increase in alveolar progenitor (AP) population, the cells of origin of TNBC, in part by CCL9[3].



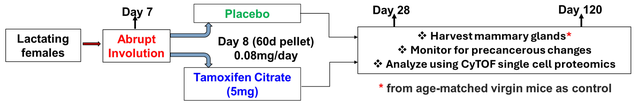
Hypothesis: In this study, we addressed the hypothesis that anti-estrogen tamoxifen (TAM) could revert the adverse effects of abrupt involution/lack of breastfeeding by deep interrogation of shifts in cellular phenotypes through CyTOF single cell proteomics[4].

METHODS

Mouse model: Uniparous **wild type FVB/N** mice (~12 weeks old) with 6pups/dam were assigned to Gradual Involution (GI) or Abrupt Involution (AI) cohorts. **GI:** pups were weaned on postpartum (PP) day 28 and 31. **AI:** all pups were weaned on PP day 7.



Tamoxifen treatment: Sustained release Tamoxifen Citrate pellets (5mg) or placebo was implanted in the subscapular region of the dams on PP day 8. Mammary glands were harvested on PP day 28 & 120 for subsequent use.



Tamoxifen abolishes AI-induced precancerous changes

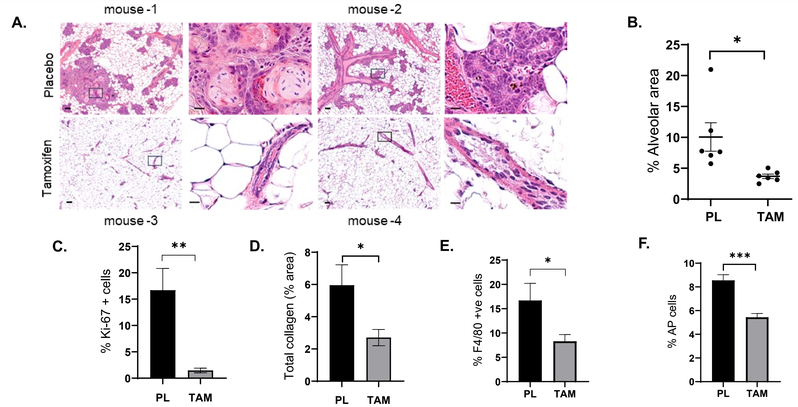


Figure 1. (A) H&E stained sections of mouse mammary gland showing histological differences. **(B)** Graph showing percentage of alveolar area in the mammary glands. **(C-E)** Immunohistochemical staining of mammary gland sections with Ki67 antibody **(C),** trichrome **(D),** and F4/80 antibody **(E),** quantification presented in the bar diagrams. **(F)** Percentage of the Alveolar Progenitor (AP) cells mammary epithelial cells in glands harvested on day120 PPM, under the two treatment conditions. (PL: Placebo, TAM: Tamoxifen). * p<0.05, **, p<0.01, ***, p<0.001.

Tamoxifen restores long-term lineage balance after AI

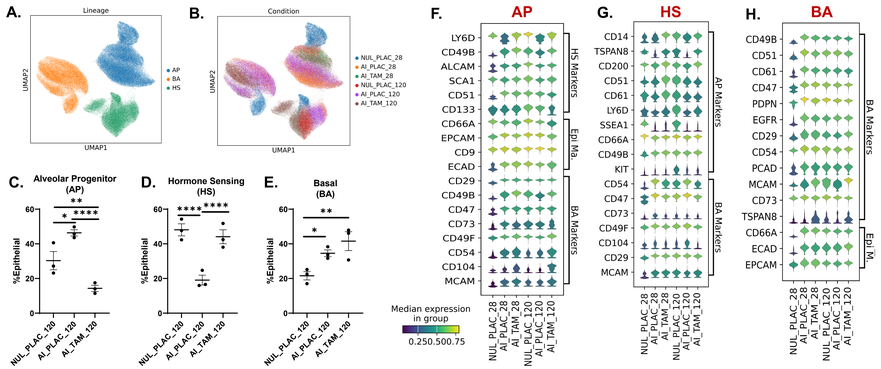
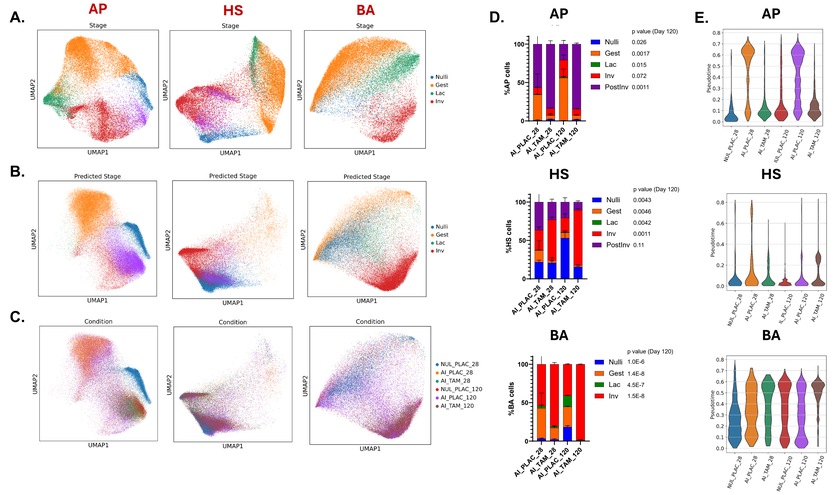


Figure 2. (A-B) UMAPs showing the epithelial lineage **(A)** and condition **(B)** of the epithelial cells analyzed by CyTOF. **(C-E)** Graphs showing the percentages of epithelial cells in each 120-day condition that are in the AP **(C)**, HS **(D)**, and BA **(E)** lineages. *, p<0.05, **, p<0.01, ****, p<0.0001. **(F-H)** Violin plots showing the expression of markers differentially expressed between conditions in AP **(G)**, HS **(H)**, and **BA (I)** cells. Marker expression is scaled between 0 and 1.

Tamoxifen alters differentiation marker expression in epithelial cells



(A) UMAPs showing the lineage trajectories for AP (left), HS (middle), and BA (right) cells in a typical differentiation cycle, colored by the stage at which cells were collected. **(B-C)** UMAPs showing the integration of the cells from Figure 2 into the lineage trajectories from **(A)**, colored by predicted stage **(B)** or by condition **(C). (D)** Bar charts showing what percentage of cells from each AI condition were assigned to each stage of the differentiation cycle. Significance was determined by the ScanPro ANOVA for complex compositional analyses with follow-up pairwise t-tests. The adjusted p-values for the AI_PLAC_120 and AI_TAM_120 comparisons are shown to the right of each chart. **(E)** Violin plot showing the pseudotime for the cells from each condition.

Conclusions

This work demonstrates that tamoxifen offers marked protection against abrupt involution induced precancerous changes in the mouse mammary glands, restoring long-term lineage balance and altering differentiation of the alveolar progenitor cells towards a typical post-involution state as opposed to abrupt involution-induced hyperplasia. The corrective effect of TAM provides a rationale for short-term use of tamoxifen as a preventive measure for women who are unable to breastfeed to reduce risk of breast cancer

References

1. *Lancet 360, 187-95 (2002).PMID: 12133652*
2. *Basree, M.M., Shinde, N., Koivisto, C. et al. Breast Cancer Res 21: 80 (2019). PMID: 31315645*
3. *Majumder s., Mishra S., Shinde, N. et.al. Breast Cancer Res 21: 80 (2024). PMID: 39754221*
4. *Gray, G.K., Girnius, N, Kuiken, H. J., et. al. Cell Report 42(10): 113293 (2023). PMID: 3785468*

This study is supported by : RO1 CA 231857 to BR/RG and in part by an NCI F31 CA22820 to GKG