Investigating tumor promotion in the postpartum liver metastatic niche

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Background

- Postpartum breast cancer (PPBC) is defined as breast cancer diagnosed within 10 years of a pregnancy. PPBC patients are more likely than their never-pregnant (nulliparous) counterparts to develop liver metastases (Figure 1)
- Metastasis seems to be driven by host biology, as poor prognosis is independent of biologic subtype, tumor size, age at diagnosis, and year of diagnosis

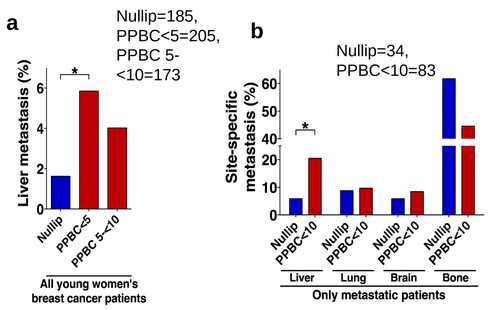
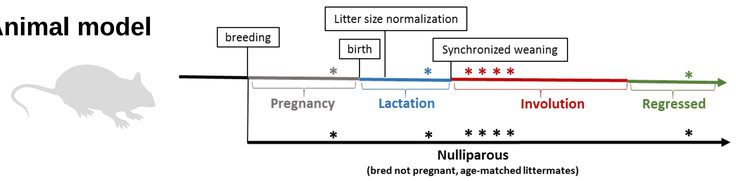


Figure 1. a) Frequency of liver metastasis in nulliparous and PPBC patients from a University of Colorado (UCH) cohort, N=563; p=0.03; Logistic regression multivariate analysis. **b)** Frequency of liver (p=0.04, OR: 4.12), lung (p=1.00, OR: 1.10), and bone metastasis (p=0.11, OR: 0.50) in a combined young women's breast cancer cohort from UCH and Dana-Farber Cancer Institute (DFCI) demonstrates tissue-tropism for metastases to the liver, N=117; Fisher's exact. Goddard et al, 2017.

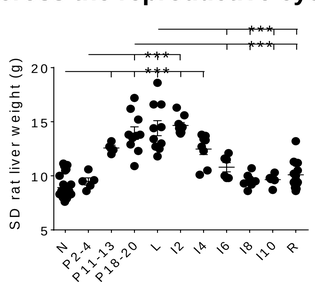
The liver in postpartum young women preferentially supports liver metastasis.

Unique postpartum liver biology that may facilitate metastasis: weaning-induced liver involution

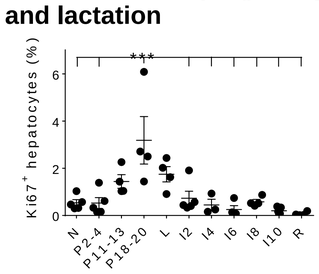
a) Animal model



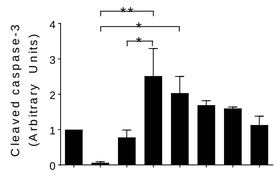
b) Change in liver weight across the reproductive cycle



c) Proliferation drives liver size increase in pregnancy and lactation



d) Cell death drives liver weight loss post-weaning



e) Liver metabolism supports liver expansion and involution

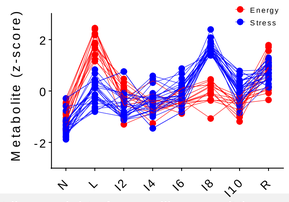


Figure 2. a) Breeding schema. **b)** Sprague-Dawley (SD) rat liver weights from nulliparous (N), pregnancy (P), lactation (L), days 2, 4, 6, 8, 10 post-weaning (involution, Inv2, Inv4, etc), and regressed stages (R). **c)** Ki67 positive rat hepatocytes **d)** Cleaved caspase-3 western blot from pooled liver lysates. **e)** Metabolite z-scores showing an energy metabolism signature detected by mass-spectrometry at lactation and a cell stress signature at involution day 8 in rat livers (average of n=4-6 rats/grp). * p: <0.05, ** p:<0.01, *** p: <0.001, One-way ANOVA (Goddard et al, February 2017)

Mouse portal vein injection model reveals increased liver metastasis post-weaning, matching PPBC data

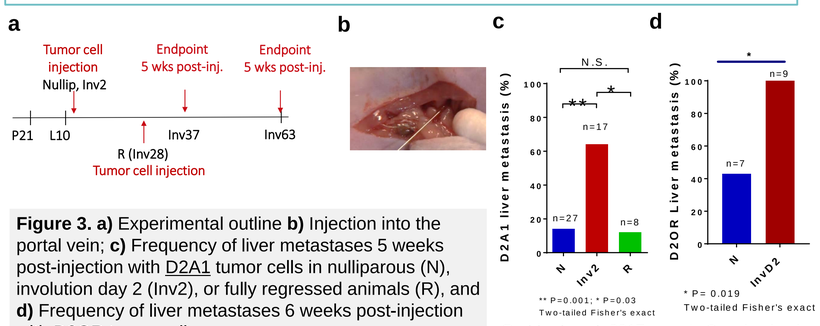


Figure 3. a) Experimental outline **b)** Injection into the portal vein; **c)** Frequency of liver metastases 5 weeks post-injection with D2A1 tumor cells in nulliparous (N), involution day 2 (Inv2), or fully regressed animals (R), and **d)** Frequency of liver metastases 6 weeks post-injection with D2OR tumor cells.

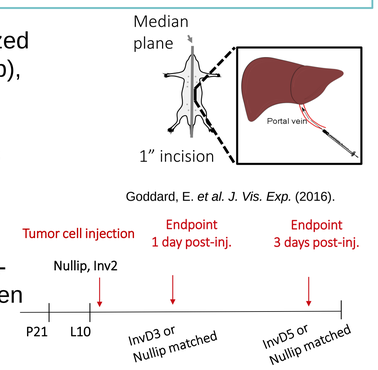
Goddard et al, 2017 A. Quackenbush

Research Questions

1. What steps of the metastatic cascade are supported by the involuting liver?
2. What features of the involuting liver provide an advantage to metastatic tumor cells?

Experimental outline: Metastatic cascade question

1. Use Glowing Head Mice, which are tolerized to GFP protein (transgenic Gnhrh-luc-egfp), allows for use of GFP-tagged cells
2. GFP-tagged D2OR mammary tumor cells are injected via portal vein injection into nulliparous or involution day 2 mice
3. Mice are euthanized at 1 and 3 days post-injection to study early interactions between tumor cells and liver environment



Reproductive state impacts tumor cell phenotype in liver at early time points after tumor cell injection

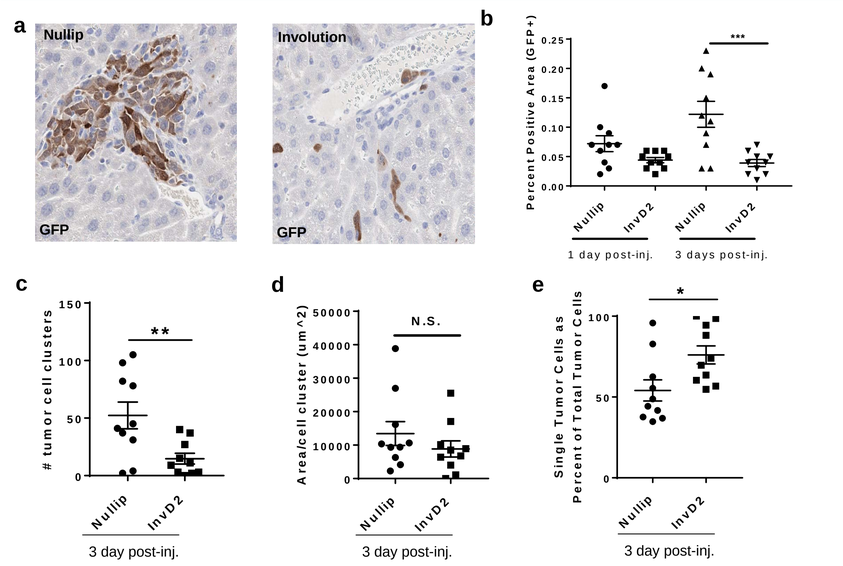
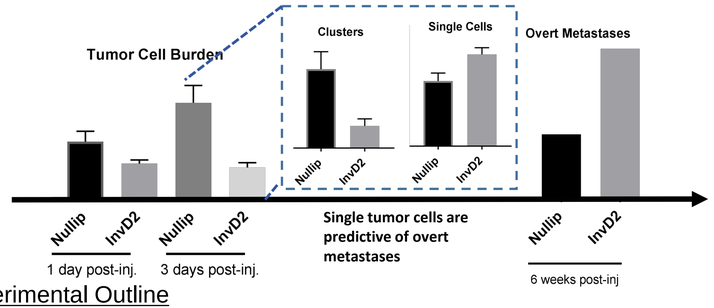


Figure 4. a) Representative images from liver tissue stained by IHC for GFP to detect D2OR-GFP tumor cells injected via the portal vein; **b)** Quantitative IHC for D2OR-GFP tumor cells injected into nulliparous or involution day 2 host livers with mice sacrificed 1 and 3 days after tumor cell injection, Tukey's Multiple Comparisons Test p<0.001; **c and d)** Counts and average size of tumor cell clusters and **e)** single tumor cells found in livers of nulliparous or involution day 2 mice injected with D2OR-GFP tumor cells and sacrificed 3 days post-tumor cell injection; Student's t test, * p: <0.05, ** p:<0.01

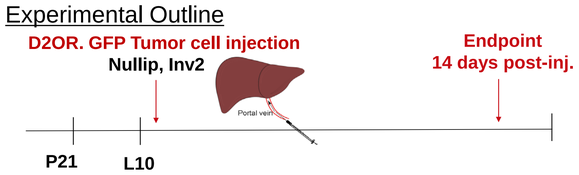
Future (ongoing) direction: Assess if involution metastatic promotion emerges by day 14 post tumor cell injection

Hypothesis: Metastatic advantage in involution may be immune-mediated; single tumor cells may preferentially avoid immune clearance.

Premise: The involution liver environment is enriched for immune suppressive features that may promote metastasis.



Experimental Outline



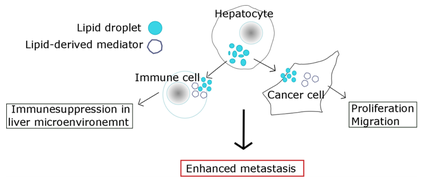
14 days time point chosen because it allows observation of result of possible immune-mediated tumor cell clearance

Potential mediators of immune suppression in the involution liver microenvironment

Candidate mediators include pro-tumor extracellular matrix proteins and an axis that is predicted to facilitate immune suppression:

- Suppressive myeloid cells
- FoxP3+ T regulatory cells
- Elevated TGFβ mRNA

My preliminary data identify a new feature of the involution liver environment that may contribute to metastatic promotion: lipid accumulation



Lipid droplet accumulation in the periportal zone is a new feature of weaning-induced involution

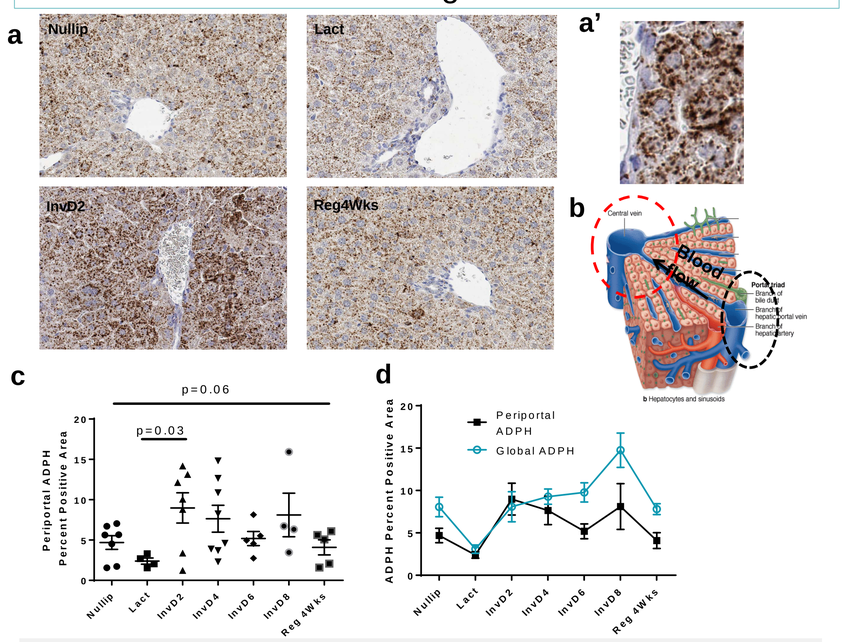


Figure 7. a) Representative adipophilin-stained IHC mouse liver sections from nulliparous (Nullip), lactation (Lact), involution day 2 (InvD2), and regressed (Reg4Wks) reproductive stages. **a')** Magnified view of ADPH punctate stain pattern. **b)** Depiction of liver anatomical organization shows portal triad ['periportal'] zone (Junqueira's Basic Histology, McGraw Hill) **c)** Quantification of periportal adipophilin-stained area reveals specific early accumulation of lipids in the periportal zone post-weaning; One-way ANOVA. **d)** Overlay of periportal and total (i.e. global) adipophilin-stained area demonstrates differential lipid droplet localization across reproductive stages.

Lipidomics reveals reproductive state-dependent regulation of hepatic lipid content

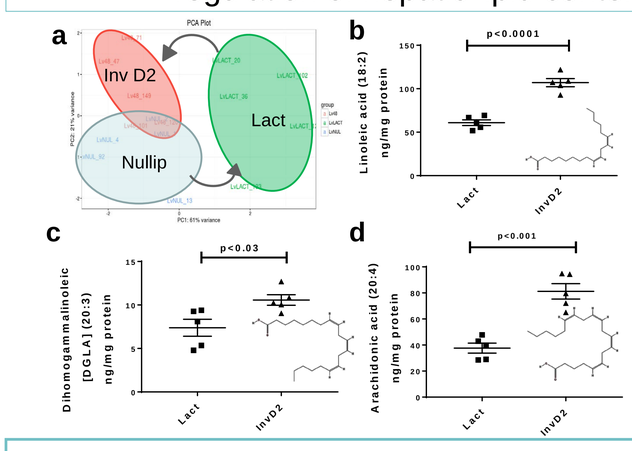


Figure 8. a) Principal component analysis illustrates distinct lipid profiles across reproductive stage, n=5/group. **b-d)** Absolute abundance of select unsaturated fatty acids from whole liver tissues lactation and involution day 2 mice (n=5/group); all p-values: unpaired Student's T-test

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