Biomimetic Scaffolds Recapitulate Anti-Tumor Phenotypes in the Early Breast Cancer Metastatic Niche

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Introduction

One in eight women are diagnosed with breast cancer in their lifetime[1]

Five-year survival dependent on disease stage[2]
- 99% - localized disease
- 27% - metastatic disease

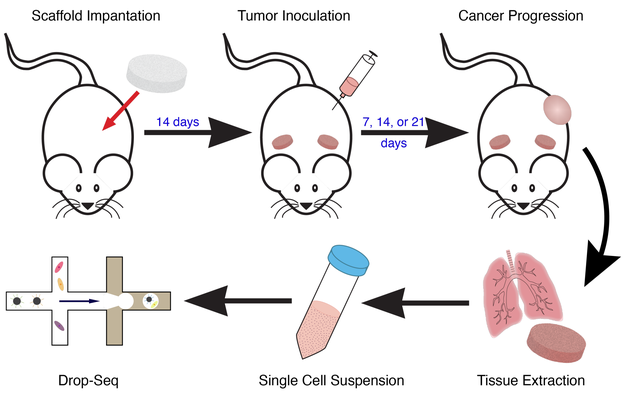
Breast cancer metastasis often[1]
- found at the lungs, liver, bone, brain
- not detected until radiological evidence
- incurable

Metastatic niche initiates in healthy tissue before progressing to pre-metastatic and then overt metastatic; dynamics of developing niche are poorly understood

We use a biomaterial scaffold that acts as synthetic pre-metastatic niche[3-5]

Methods

Porous polycaprolactone scaffolds (5 mm x 2 mm) were implanted subcutaneously in Balb/c mice



Seurat	Analysis of single cell data
GSEA	Differential signaling pathways
DoRothEA	Prediction of transcription factor activity
CellPhoneDB	Receptor-ligand interactions

Conditioned medium generated in a 24 h culture (0.3 mg/ml) and used 1:1 with tumor cell medium for phenotypic assays.

References

1. Susan G. Komen Breast Cancer Foundation
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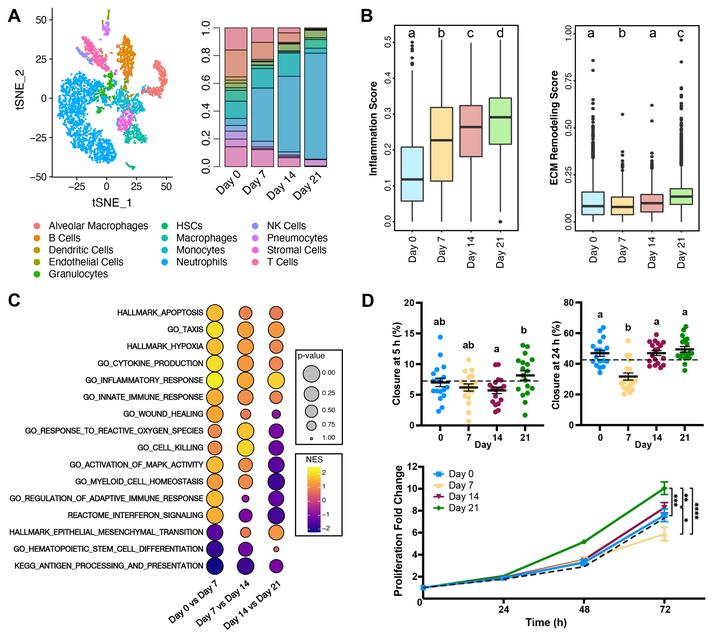
3. Bushnell, G.G. *et al.*, Cancer Res, 2019.
4. Oakes, R.S. *et al.*, Cancer Res, 2020.
5. Bushnell, G.G.* and S.M. Orbach* *et al.*, Biomaterials, 2020.

Anti-Tumor Signaling in the Lung Pre-Metastatic Niche



Day 0: Healthy
Day 7: Pre-Metastatic
Day 14: Early Metastatic
Day 21: Metastatic

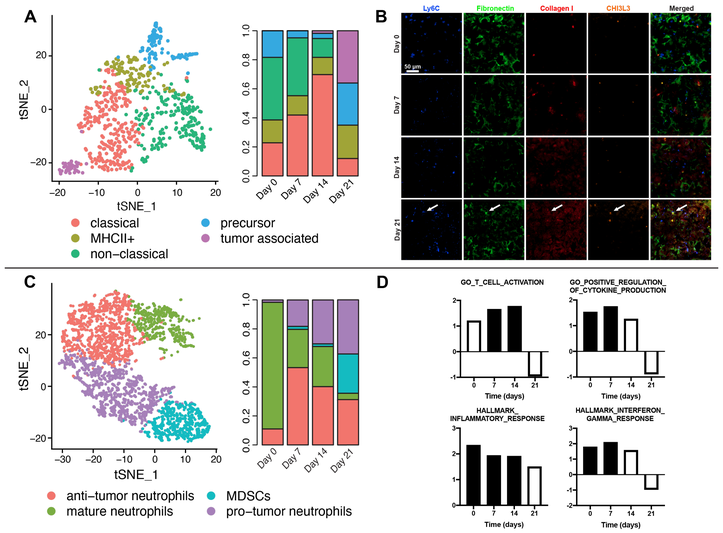
(A) Cell populations at the lung metastatic niche are highly dynamic – marked by increases in neutrophils. **(B)** Signaling pathways associated with the hallmarks of cancer increased over time. **(C)** Unsupervised GSEA analysis shows a transition from pro-inflammatory/anti-cancer signaling to immune cell dysfunction between days 7 and 21. **(D)** Day 21 conditioned medium induced tumor cell proliferation and migration, while day 7 conditioned medium inhibited these responses.

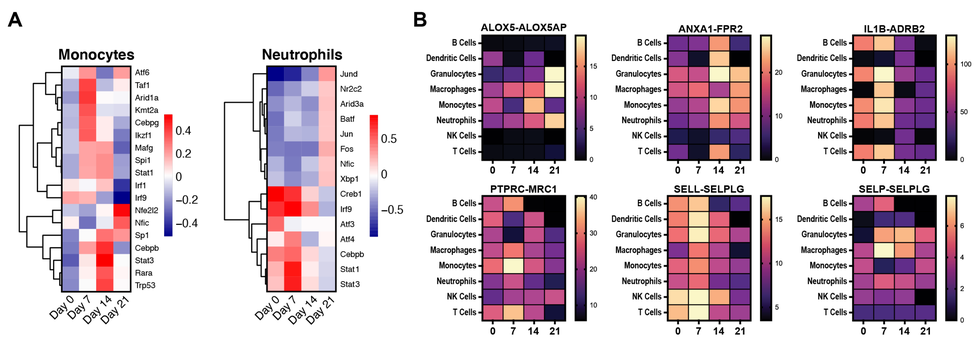
Despite evolving cell dynamics implicating worsening disease, the pre-metastatic niche has an anti-cancer microenvironment

Monocyte- and Neutrophil-Driven Phenotypic and Signaling Dynamics

(A) Tumor-associated monocytes (Chi3l3+ classical monocytes) only appear at the metastatic niche at day 21. **(B)** Excess extracellular matrix proteins are localized around monocytes at day 21, particularly tumor-associated monocytes. **(C)** Over two-thirds of neutrophils at days 7 and 14 maintain anti-cancer phenotypes. **(D)** Early neutrophils are up-regulated for pro-inflammatory (anti-cancer) responses, while the same pathways are down-regulated in late neutrophils, black bars are significant with $p < 0.05$.

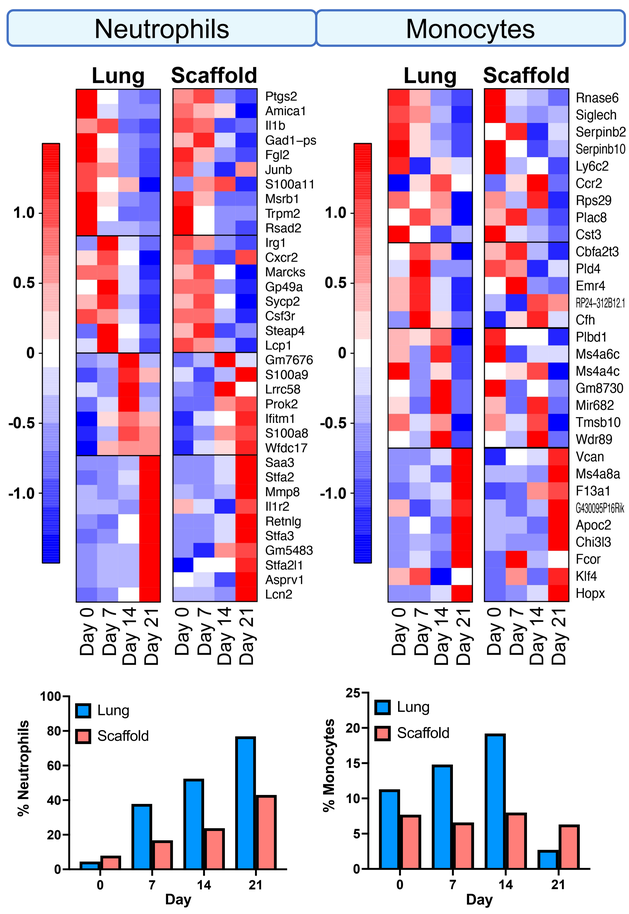
Phenotypic changes in monocytes and neutrophils drive the anti- to pro-tumor transition in the lung





(A) Monocyte and neutrophil active transcription factors are anti-tumor at days 7 and 14, and pro-tumor at day 21. **(B)** Neutrophil-specific ligands interact with receptors on all immune cell types and influence the transition from anti- to pro-cancer phenotypes.

Biomimetic Scaffolds



Genes that correlate with lung neutrophil and monocyte phenotypic evolution follow the same trends in the scaffold

Conclusions

The lung metastatic niche was marked by an increase in neutrophils and expression of the hallmarks of cancer

Cells in the microenvironment of the pre-metastatic niche have an anti-tumor phenotype driven by the monocytes and neutrophils

Monocyte and neutrophil phenotypes in the biomimetic scaffolds recapitulate their counterparts in the native lung throughout disease progression

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