Neutrophil Elastase promotes myoepithelial layer dissolution and breast cancer progression

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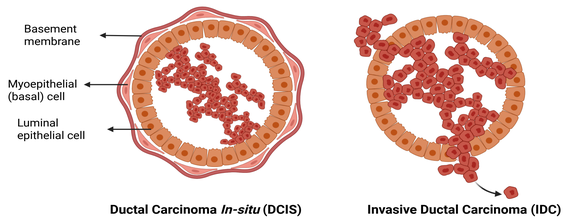
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Abstract

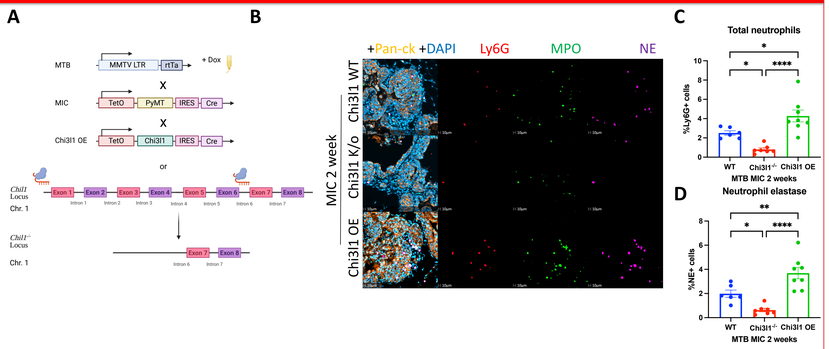
Progression from ductal carcinoma *in-situ* (DCIS) to invasive ductal carcinoma (IDC) underlies metastatic breast cancer, yet no biomarkers exist to distinguish DCIS lesions that will progress to IDC. In this study, we uncover an immunological driver of this transition. In two independent breast-cancer mouse models, neutrophil depletion or genetic deletion of their chemoattractant Chitinase-3 like 1 (Chi3l1) preserved the myoepithelial barrier, halted DCIS-to-IDC transition and reduced lung metastasis. Conversely, Chi3l1 overexpression increased neutrophil infiltration, accelerated myoepithelial loss and promoted dissemination, phenotypes reversed by neutrophil depletion. Human DCIS samples with high neutrophil levels similarly showed myoepithelial disruption. Mechanistically, tumor-infiltrating neutrophils secrete neutrophil elastase (NE) which promotes myoepithelial layer dissolution. Pharmacological NE inhibition restored myoepithelial integrity and blocked DCIS progression. These data demonstrate that neutrophils contribute to DCIS progression through NE-mediated loss of the myoepithelium. Our findings position neutrophils, Chi3l1 and NE as tractable biomarkers and therapeutic targets for high-risk DCIS.

INTRODUCTION INTO THE MYOEPITHELIAL LAYER



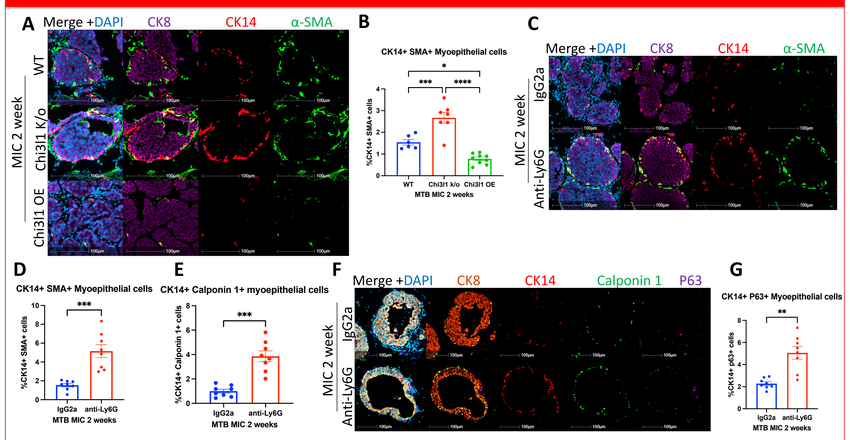
- Myoepithelial cells form a layer around luminal cells.
- They possess tumor-suppressive functions and play a protective role against tumor growth.
- Loss of the myoepithelium is a key stage during breast cancer progression.
- Myoepithelial markers: CK14, Alpha-SMA, Calponin 1, p63.
- Neutrophils are known to degrade the ECM and basement membrane but their impact on the myoepithelial layer is unknown.
- *The goal of this project is to investigate the functional contribution of neutrophils to myoepithelial layer dissolution and DCIS progression.*

Chi3l1 is a neutrophil chemoattractant



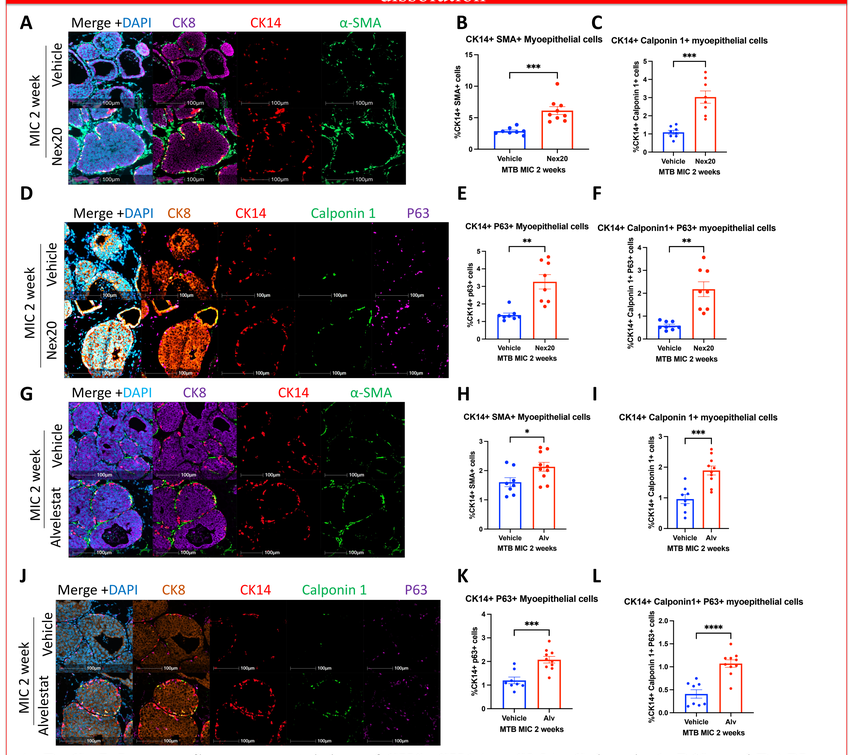
A. Schematic summary of the Chi3l1 knockout (Chi3l1 k/o) or mammary epithelial-specific Chi3l1 overexpression (Chi3l1 OE) mouse models used in this study. These mice were crossed with our inedible PolyomaVirus middle T breast cancer mouse model (MIC Model). Figure created using Biorender.com. **B.** Immunofluorescent staining of Ly6G, MPO, NE, Pan-ck and DAPI on MIC WT, Chi3l1 k/o and Chi3l1 OE mammary lesions. **C,D.** Quantification of Ly6G+ and NE+ cells.

Neutrophils promote myoepithelial layer dissolution



A. Immunofluorescent staining of CK8, CK14, α-SMA, and DAPI in WT, Chi3l1 k/o and Chi3l1 OE mammary glands. **B.** Quantification of CK14+ SMA+ cells. **C, F.** Immunofluorescent staining of CK8, CK14, α-SMA, Calponin 1, P63, and DAPI on Anti-Ly6G-treated MIC WT mammary lesions. **D, E, G.** Quantification of CK14+ SMA+, CK14+ Calponin 1+ and CK14+ P63cells.

Neutrophil elastase released during degranulation leads to myoepithelial layer dissolution



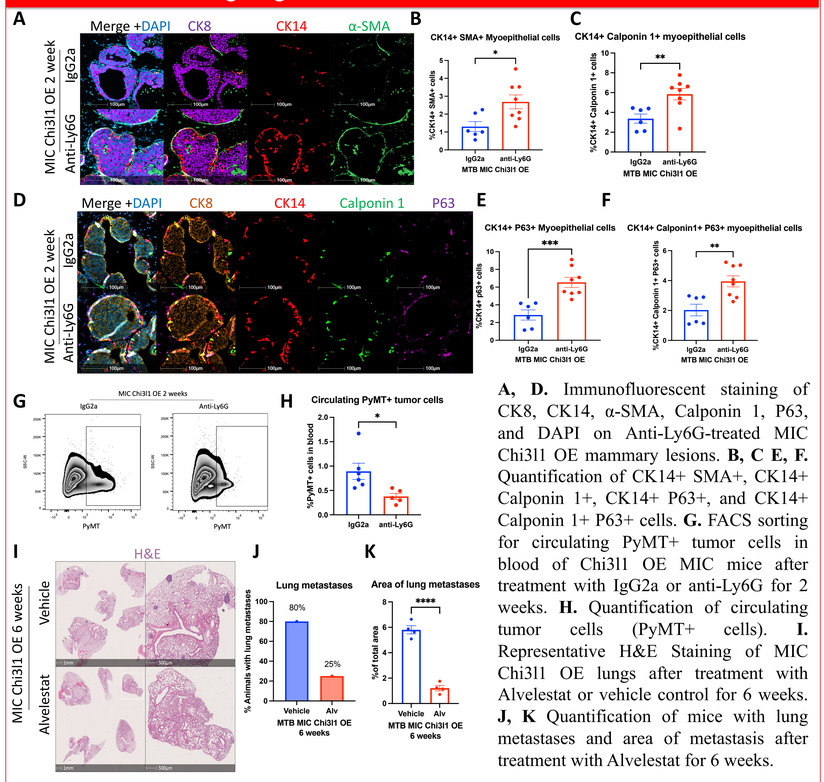
A, D, G, J. Immunofluorescent staining of CK8, CK14, α-SMA, Calponin 1, P63, and DAPI on Nex20-, Alvelestat-, and vehicle-treated MIC WT mammary lesions. **B, C, E, F, H, I, K, L.** Quantification of CK14+ SMA+, CK14+ Calponin 1+, CK14+ P63+, and CK14+ Calponin 1+ P63+ cells. *Nexinhib 20 (Nex20) selectively inhibits neutrophil degranulation. Alvelestat (Alv) specifically inhibits NE.

Conclusions and future directions

- Neutrophils play a key role in DCIS to IDC transition.
- Neutrophils and neutrophil chemoattractants are potential targets and biomarkers for DCIS progression.
- Mechanistically, Neutrophils release NE which degrades the basement membrane, leading to myoepithelial layer anoikis.

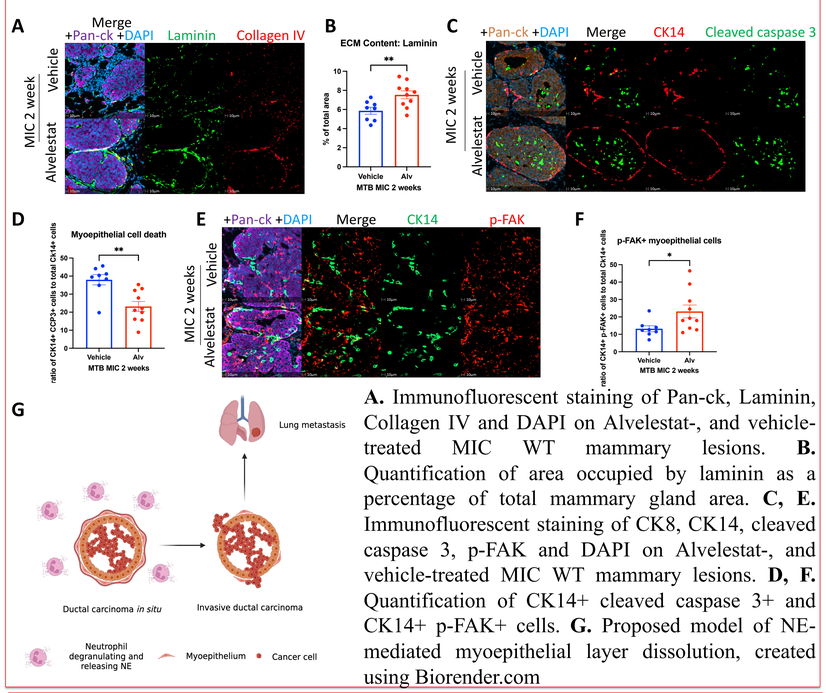
Future directions: Interrogate the clinical relevance of our findings in patient samples: Immunostain human DCIS tissues for Chi3l1 and NE and perform correlation analyses on their disease progression.

Targeting NE inhibits metastatic dissemination



A, D. Immunofluorescent staining of CK8, CK14, α-SMA, Calponin 1, P63, and DAPI on Anti-Ly6G-treated MIC Chi3l1 OE mammary lesions. **B, C E, F.** Quantification of CK14+ SMA+, CK14+ Calponin 1+, CK14+ P63+, and CK14+ Calponin 1+ P63+ cells. **G.** FACS sorting for circulating PyMT+ tumor cells in blood of Chi3l1 OE MIC mice after treatment with IgG2a or anti-Ly6G for 2 weeks. **H.** Quantification of circulating tumor cells (PyMT+ cells). **I.** Representative H&E Staining of MIC Chi3l1 OE lungs after treatment with Alvelestat or vehicle control for 6 weeks. **J, K** Quantification of mice with lung metastases and area of metastasis after treatment with Alvelestat for 6 weeks.

Targeting NE inhibits myoepithelial layer anoikis



A. Immunofluorescent staining of Pan-ck, Laminin, Collagen IV and DAPI on Alvelestat-, and vehicle-treated MIC WT mammary lesions. **B.** Quantification of area occupied by laminin as a percentage of total mammary gland area. **C, E.** Immunofluorescent staining of CK8, CK14, cleaved caspase 3, p-FAK and DAPI on Alvelestat-, and vehicle-treated MIC WT mammary lesions. **D, F.** Quantification of CK14+ cleaved caspase 3+ and CK14+ p-FAK+ cells. **G.** Proposed model of NE-mediated myoepithelial layer dissolution, created using Biorender.com

Acknowledgments

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CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

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