Fatty Acid Exposure Promotes Age-Related Mammary Tissue Alterations with Pro-Tumorigenic Potential

Mariana Bustamante Eduardo[1], Abul B.M.M.K. Islam[2], Curtis W. McCloskey[3], Maria Paula Zappia[2], Ashok Z. Samuel[4], Maxim V. Frolov[2], Rama Khokha[5], Rohit Bhargava[4], Elizaveta V. Benevolenskaya[2], Seema A. Khan[1], Susan E. Clare[1]

[1]Northwestern University, Chicago, IL,[2]University of Illinois at Chicago, Chicago, IL,[3]University of Pittsburgh, Pittsburgh, PA,[4]University of Illinois at Urbana-Champaign, Urbana, IL, [5]Princess Margaret Cancer Centre, Toronto, ON, Canada

Introduction

- *In vitro* and *ex vivo* exposure of non-transformed breast cells and breast microstructures to medium chain (MC) fatty acids (FAs) induces a metabolic shift toward the serine, one-carbon, glycine and methionine pathways (SOG/methionine), engendering epigenetic plasticity, increasing reactive oxygen species (ROS), promoting cell survival and disrupting cell-cell communication.[1,2]
- Similarly, disrupted cell-cell communication, epigenetic plasticity and increased ROS are also reported in the aged mammary gland. [3-5]
- **We hypothesize that FA-induced metabolic reprogramming leads to biological aging of the mammary gland, contributing to pro-tumorigenic alterations observed during aging.**

Methods

- Single-cell RNA-Seq (scRNA-seq) was performed on primary human breast epithelial cells exposed to octanoic (OA). Cell-cell communication was analyzed using CellChat.
- Breast microstructures embedded in Matrigel, and 3D mammary spheres formed from primary mammary cells and embedded in Matrigel were exposed to ± OA for 7 days, followed by staining for luminal and basal markers, F-actin, laminin, mitochondria and nuclei, and imaged using confocal microscopy.
- Migratory cell populations in OA-containing media were identified through a primary breast cell culture selection assay coupled with scRNA-seq analysis. scCellFie was used for analyzing metabolic activity.
- Raman spectroscopy was used to characterize the lipid content in pre- and post-menopausal breast tissue.

References

(1) Yadav, S. *et al.* Lipid exposure activates gene expression changes associated with estrogen receptor negative breast cancer. *npj Breast Cancer* **8,** 59 (2022).

(2) Bustamante Eduardo et al. A metabolic shift to the serine pathway induced by lipids fosters epigenetic reprogramming in nontransformed breast cells.*Sci. Adv.***11**,eads9182 (2025).

(3) Yan, P. *et al.* Midkine as a driver of age-related changes and increase in mammary tumorigenesis. *Cancer cell* **42**, 1936-1954.e1939 (2024).

(4) Li, C. M.-C. *et al.* Aging-Associated Alterations in Mammary Epithelia and Stroma Revealed by Single-Cell RNA Sequencing. *Cell reports* **33** (2020).

(5) Angarola, B. L. *et al.* Comprehensive single-cell aging atlas of healthy mammary tissues reveals shared epigenomic and transcriptomic signatures of aging and cancer. *Nature Aging* **5**, 122-143 (2025).

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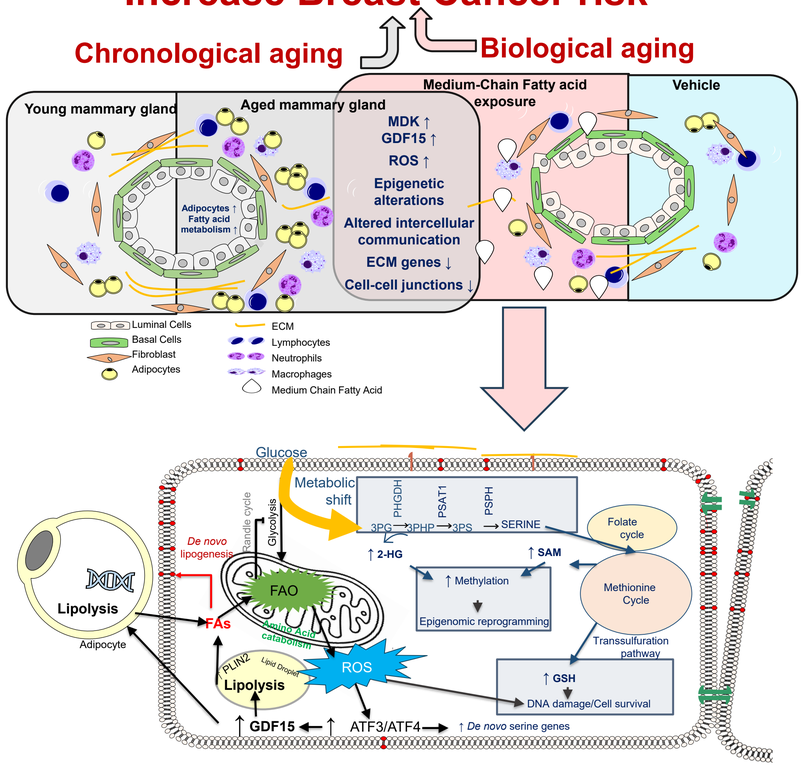
Implications

Increase Breast Cancer risk

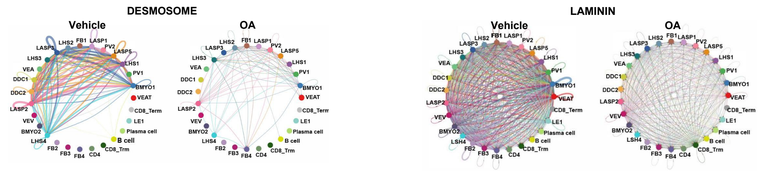
Chronological aging → Biological aging



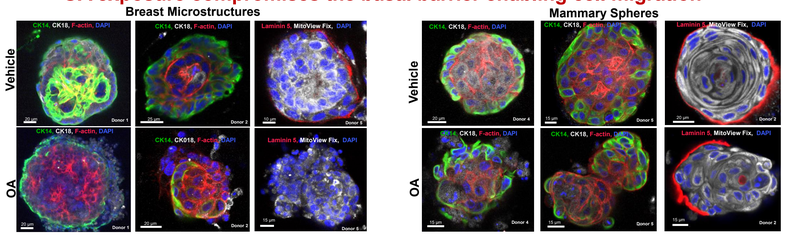
- **Medium chain fatty acids** (e.g. OA) drives **anabolic metabolic reprogramming** in breast cells supported by increased amino acid catabolism and ***de novo* lipogenesis.**
- Resulting lipid changes alter membrane composition and **destabilize tissue structure**.
- Elevated fatty acids promote mammary gland remodeling and **accelerate biological aging**.
- Chronological and biological aging **increase vulnerability to breast cancer**. These findings reveal potential preventive strategies such as targeting GDF15 and SOG/methionine.

Results

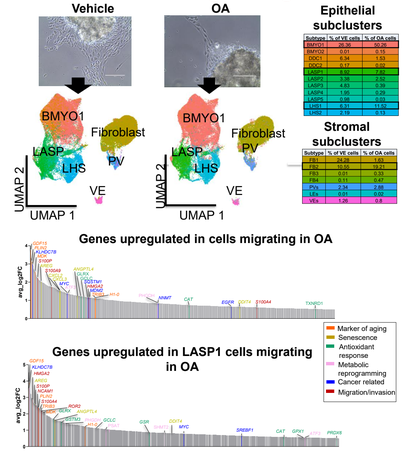
OA exposure decreases ECM-cell interactions and cell-cell adhesions



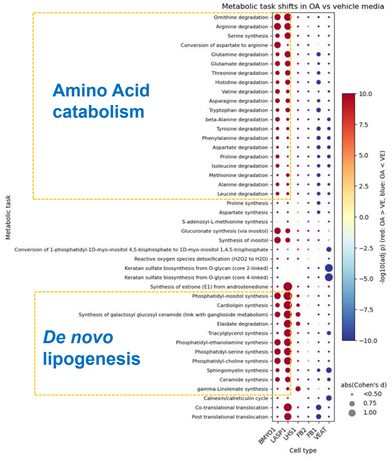
OA exposure compromises the basal barrier enabling cell migration



OA selects cells expressing aging, cancer and migration genes

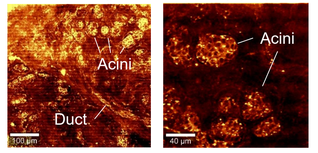


OA induces metabolic shifts in epithelial cells

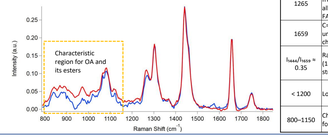


MCFAs are present in the normal breast as revealed by Raman spectroscopy

Raman images of breast tissue (Lipids)



Spectra of premenopausal (blue) and postmenopausal (red) breast tissue



Raman Band (cm^{-1})	Assignment / Origin	Observation / Ratio	Interpretation
1265	cis =C–H bending from unsaturated alkyl chains of FAs/lipids	—	Indicates presence of unsaturated FAs/lipids
1659	C=C stretching from unsaturated alkyl chains of FAs/lipids	Intensity reflects degree of unsaturation	Reliable marker of lipid unsaturation
I_{1442}/I_{1659} ≈ 0.35	Ratio of CH_2 bending (1444 cm^{-1}) to C=C stretching (1659 cm^{-1})	Similar in both tissues; lower than mono-unsaturated lipids	Indicates higher fraction of saturated alkyl chains in tissue lipids
< 1200	Low-frequency region	Enhanced intensity in postmenopausal tissue	Suggests increased contribution of medium-chain FAs
800–1150	Characteristic region for OA and its esters	Stronger Raman bands observed	Supports presence of MCFAs