Metabolic Shift Toward the *de novo* Serine Pathway in Non-Transformed Breast Cells Drives Epigenetic Plasticity, Oxidative DNA Damage, and Pro-Tumorigenic Changes Associated with Aging

Mariana Bustamante Eduardo[1], Curtis W McCloskey[2], Gannon Cottone[1], Shiyu Liu[3], Flavio R. Palma[1], Maria Paula Zappia[4], Abul B.M.M.K. Islam[4], Elizaveta V. Benevolenskaya[4], Maxim V. Frolov[4], Jason Locasale[3], Marcelo G. Bonini[1],Rama Khokha[2] Navdeep S. Chandel[1], Seema Khan[1], Susan Clare[1]
[1]Northwestern Univ. Feinberg School of Medicine, Chicago, IL, [2] Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada. [3]Duke University, Durham, NC, [4]University of Illinois at Chicago, Chicago, IL

Introduction

- Understanding the genesis of sporadic estrogen receptor negative breast cancer (ERneg BC) is a significantly unmet clinical need.
- Genes involved in **lipid metabolism** are overexpressed in the contralateral unaffected breast of women with ERneg BC (1).
- Exposure of non-transformed breast epithelial cells to lipids results in significant changes in metabolic flux, histone post-translational modifications and gene expression. The upregulated genes are involved in neural pathways and stemness (2).
- The association of the serine, one-carbon, glycine (SOG) and ERneg BC was first observed over a decade ago (3).
  - **We hypothesized that the metabolism of lipids in preference to glucose and glutamine results in a metabolic shift toward the SOG and methionine pathways facilitating the genesis of ERneg BC.**

Methods

- Proteomics and 13C-glucose tracing was performed in MCF-10A cells exposed to octanoic acid (OA). Targeted metabolomics was performed in MCF-10A cells exposed to OA ± PHGDH inhibitor.
- ROS-induced redox changes were monitored using ORP1-roGFP2 based sensors in MCF-10A cells
- Alkaline comet assay was done to detect DNA breaks.
- CUT&RUN for H3K4me3 was performed in MCF-10A exposed to OA. MACS2, DiffBind and ChIPseeker were used to call and annotate peaks. HOMER was used for Transcription factor binding motif enrichment analysis.
- Single-cell RNA-Seq was performed on primary human breast epithelial cells exposed to OA. Metabolic flux analysis was performed using Compass. Cell-cell communication was analyzed using CellChat and Single Cell Pathway Analysis (SCPA) was used for pathway

References

(1). Wang, J. *et al.* Overexpression of lipid metabolism genes and PBX1 in the contralateral breasts of women with estrogen receptor-negative breast cancer. *Int J Cancer* 140, 2484-2497, (2017).

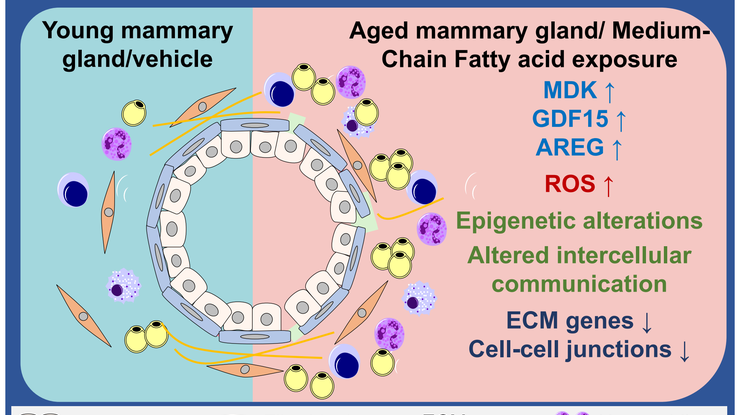
(2). Yadav, S. *et al.* Lipid exposure activates gene expression changes associated with estrogen receptor negative breast cancer. *npj Breast Cancer* **8,** 59 (2022).

(3) Possemato, R. *et al.* Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 476, 346-350 (2011).

(4) 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).

Metabolism of lipids in preference to glucose and glutamine results in a metabolic shift toward the SOG and methionine with implications for oncogenesis.

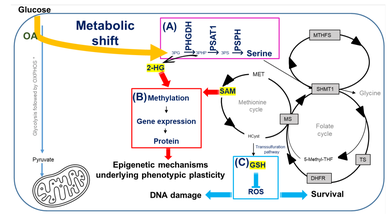
This shift leads to changes that are associated with the aging of the mammary gland that may facilitate carcinogenesis.



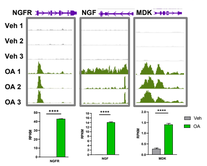
We propose that the remodeling of the mammary gland associated with aging is partly due to the increase in adipocytes and FA release.

Results

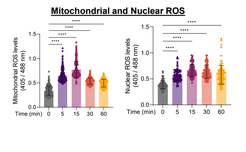
Metabolism of lipids results in a metabolic shift toward the de novo serine pathway (SSP) increasing the production of SAM, 2-HG, and GSH which have implications for oncogenesis (4)



(B) SAM and 2-HG leads to epigenetic reprogramming

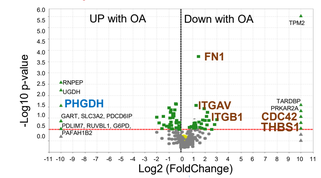


(C) Metabolic shift to SSP boosts GSH to Control ROS

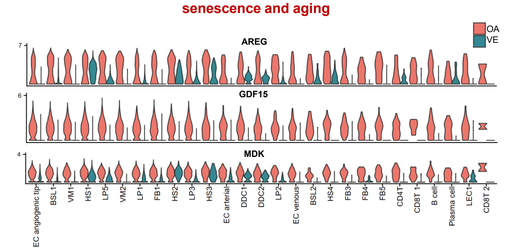


Medium chain fatty acid exposure causes changes typical of the aged mammary gland

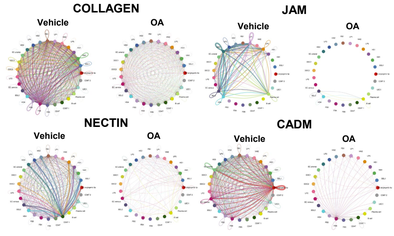
OA exposure downregulates proteins involved in ECM-receptor interaction and focal adhesion



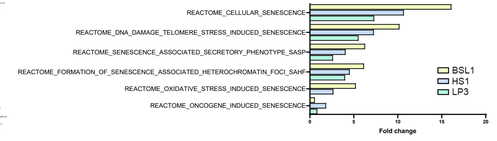
OA exposure modulates the expression of genes involved in senescence and aging



ECM-cell interactions and cell-cell adhesions decrease following OA exposure

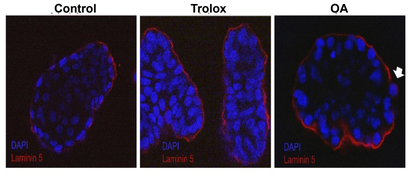


Upon OA terms related to senescence were upregulated

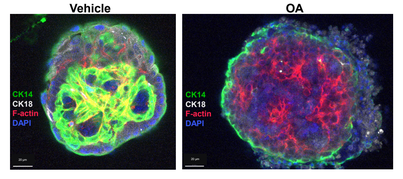


Medium chain fatty acid exposure facilitate cell invasion

Upon OA exposure the basement membrane breached



OA exposure enhances luminal cell dissemination



Research supported by the 2023 AACR-Pfizer Breast Cancer Research Fellowship; Grant Number 23-40-49-BUST.