Lipid exposure re-wires cellular metabolism away from glycolysis toward the serine pathway conferring oncogenic properties to non-transformed breast cells

Mariana Bustamante Eduardo[1], Gannon Cottone[1], Shiyu Liu[2], Flavio R. Palma[1], Maria Paula Zappia[3], Abul B.M.M.K. Islam[3], Elizaveta V. Benevolenskaya[3], Maxim V. Frolov[3], Jason Locasale[2], Marcelo G. Bonini[1], Navdeep S. Chandel[1], Seema Khan[1], Susan Clare[1]

[1]Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[2]Duke University, Durham, NC,[3]University of Illinois at Chicago, Chicago, IL

Introduction

- Understanding the genesis of sporadic estrogen receptor negative breast cancer (ERnegBC) is a significantly unmet clinical need.
- Genes involved in **lipid metabolism** are overexpressed in the contralateral unaffected breast of women with ERnegBC (1).
- Exposure of non-transformed breast epithelial cells to lipids results in significant changes in histone PTMs and gene expression. The upregulated genes are involved in neural pathways and stemness (2)
- In vitro, lipid exposure alters histone methylation affecting gene expression and increases flux through various metabolic reactions including those involved in serine, one-carbon, glycine (SOG) and methionine.(2).
- **We hypothesized that the metabolism of lipids in preference to glucose and glutamine results in a metabolic shift toward the serine pathway increasing S-adenosylmethionine (SAM) leading to histone methylation increases and changes in gene expression.**

Methods

- 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 or siRNA against PHGDH.
- ROS-induced redox changes were monitored unsing ORP1-roGFP2 based sensors in MCF-10A cells
- Alkaline comet assay was done to detect DNA breaks.
- Homologous recombination was studied in MCF-10A cells through restoration of luciferase activity from deleted substrates.
- 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 (TF) binding motif enrichment analysis.
- Single-cell RNA-Seq (scRNA-seq) was performed on primary human breast epithelial cells exposed to OA. The digital expression matrix file containing UMIs was analyzed with the Seurat package. Cell-cell communication was explored using CellChat and metabolic flux analysis was performed using Compass.

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, doi:10.1002/ijc.30680 (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). https://doi.org/10.1038/s41523-022-00422-0

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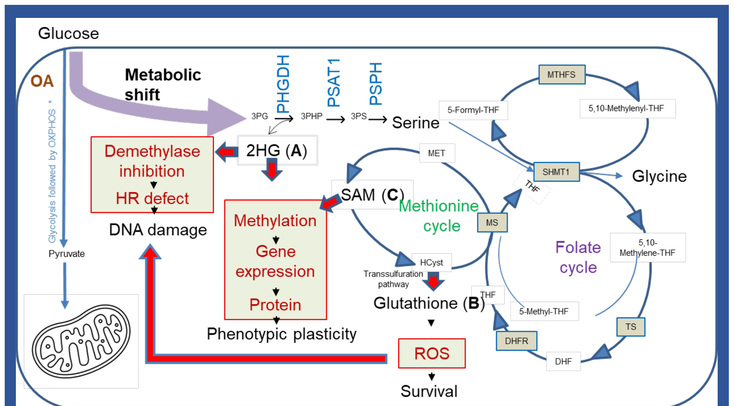
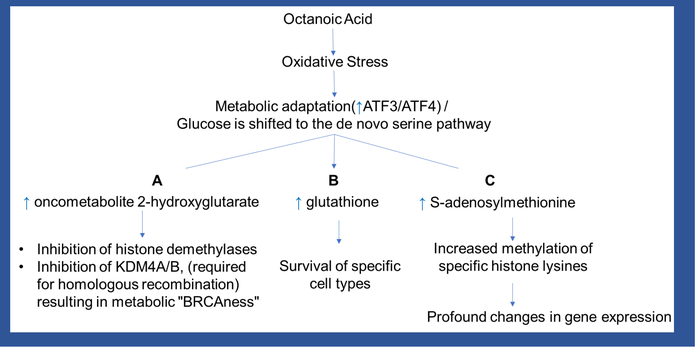


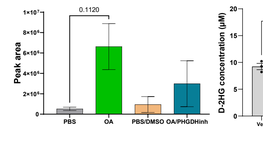
Metabolism of lipids in preference to glucose and glutamine results in a metabolic shift toward the de novo serine pathway increasing the production of 2-HG (A), glutathione (B) and SAM (C) which have implications for oncogenesis



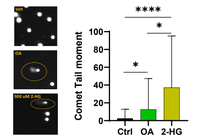
Results

A Metabolism of lipids results in a metabolic shift toward the serine pathway and increases 2-HG

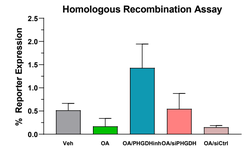
2-HG increased by OA after 30 minutes; prevented by inhibiting PHGDH



Alkaline comet assay reveals that OA and exogenous 2-HG significantly increases comet tails

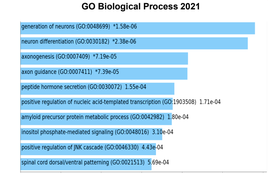


OA induced homologous recombination defect

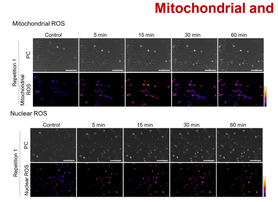


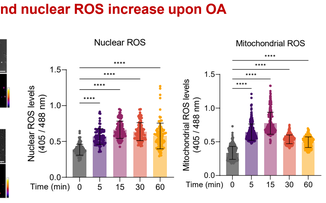
B Metabolism of lipids results in a metabolic shift toward the serine pathway increasing antioxidant defenses to control ROS

Glutathione increased by OA after 15 minutes; prevented by inhibiting PHGDH

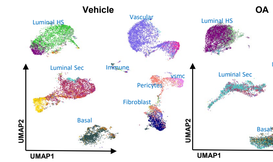


Mitochondrial and nuclear ROS increase upon OA





Upon OA treatment specific cell types increased likely reflecting the survival of cells able to mitigate oxidative stress

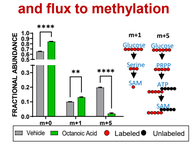


Cell subtype	% Veh	% OA
basal	10.5	11.2
LummHR-active	13.5	6.1
LummHR-major	3.5	20.3
LummHR-SCGB	2.1	3.2
Lumsec-basal	19.4	8.5
Lumsec-HLA	4.1	9.4
Lumsec-KIT	0.5	0.2
Lumsec-major	0.5	0.1
Lumsec-myo	1.0	0.8
Lumsec-prol	5.1	0.0

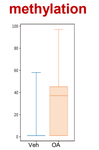
Cell subtype	% Veh	%OA
Fibro-major	0.7	4.5
Fibro-matrix	7.6	0.4
pericytes	6.2	8.2
Vas-arterial	3.32	5.54
Vas-capillary	20.90	4.65
Vas-venous	1.17	0.1
vsmc	1.60	3.22

C Metabolism of lipids results in a metabolic shift toward the serine, one-carbon and glycine (SOG) pathways increasing flux to methylation and changes gene expression

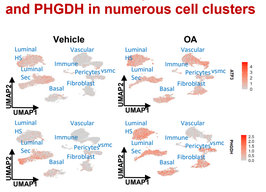
OA increased SAM M+1 and flux to methylation



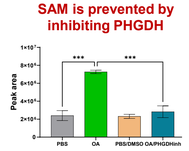
Flux to DNA methylation



OA increased the expression of ATF3 and PHGDH in numerous cell clusters



OA mediated increase in SAM is prevented by inhibiting PHGDH



OA enhanced serine metabolism and synthesis of neurotransmitters

661 differentially enriched H3K4me3 peaks upon OA mainly in promoters of neural genes