Epigenetic reprogramming induced by lipids fosters mammary cell plasticity in non-transformed breast epithelial cells

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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).
- Neural genes are highly expressed in a specific type of ERnegBC: Triple Negative Breast Cancer (3).
- **Our aim is to identify potential mechanisms linking lipids and epigenetic reprogramming to the genesis of ERnegBC**

Methods

- 13C-glucose tracing was performed in MCF-10A cells exposed to octanoic acid (OA) ± PHGDH inhibitor.
- 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.
- Gene expression was measured by qPCR in mammary microstructures derived from breast tissue exposed to OA.
- Single-cell RNA-Seq (scRNA-seq) was performed on primary human breast epithelial cells exposed to OA. The digital expression matrix file containing UMIs will be analyzed with the Seurat package version 2.3.4 R version 3.5.3 The Aldefluour assay was used to identify stem-like (ALDH+) cells upon OA.
- To determine if lipid-exposed cells adopt a neural-like phenotype, cells were grown on Poly-D-Lysine/Laminin (PDL/LM) coated plates.

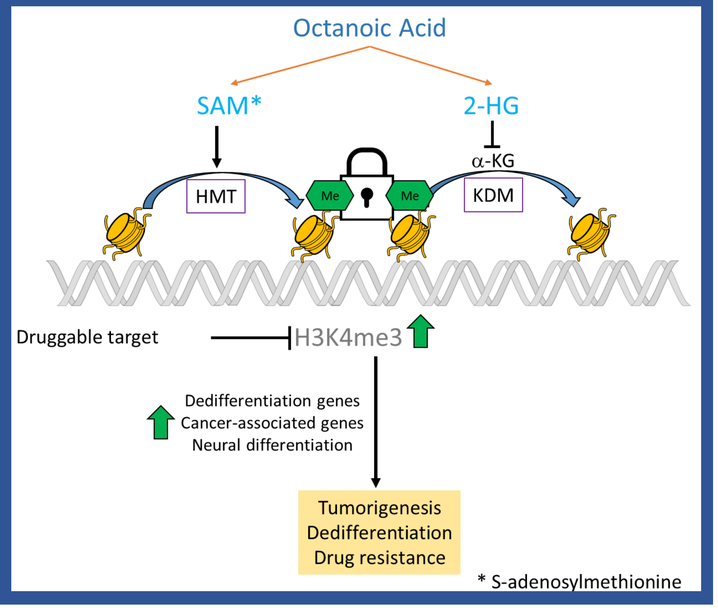
References

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

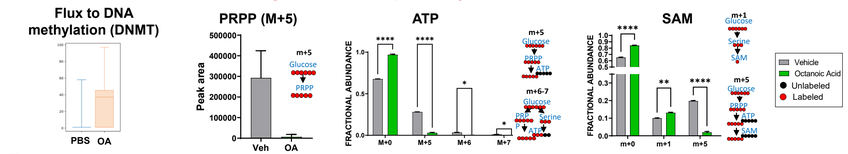
3. Tan, R. *et al.* Neural Functions Play Different Roles in Triple Negative Breast Cancer (TNBC) and non-TNBC. *Sci Rep* **10**, 3065 (2020). https://doi.org/10.1038/s41598-020-60030-5

Lipid exposure increases the production of **SAM** and **2-HG** resulting in **epigenetic fostered plasticity**, which selects cells with a multi-potential **embryonic or stem-like state** and reprograms differentiation to a **neural/neural crest-like state**

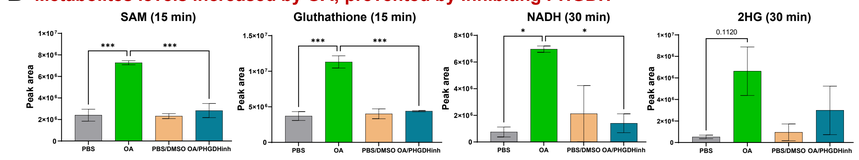


Results

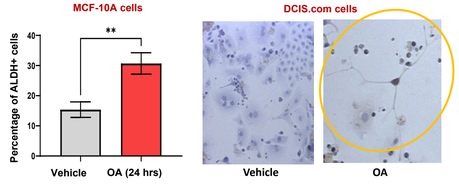
A **OA decreased flux through the pentose phosphate pathway (PPP) & increased fractional abundance of SAM M+1 through the serine pathway**



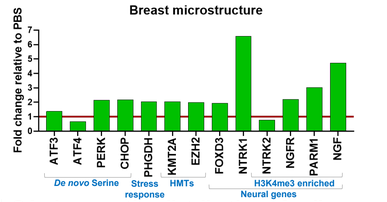
B **Metabolites levels increased by OA; prevented by inhibiting PHGDH**



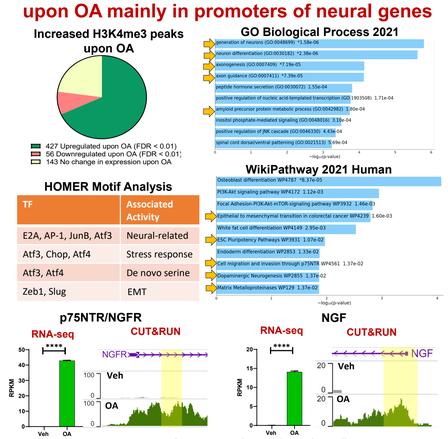
C **OA enriched ALDH+ cells and cells develop a neural-like phenotype**



D **Exposure of breast microstructure to OA changes gene expression**



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



F **OA changes the distribution of cell subpopulations (e.g. LP3) and modulates gene expression within each subcluster (e.g. BSL1)**

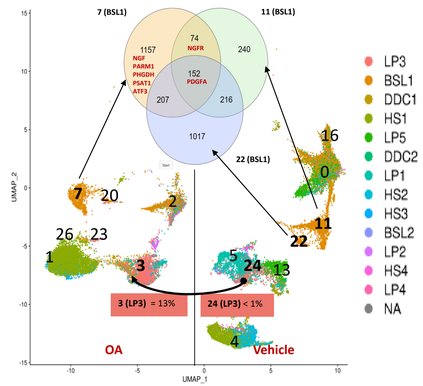


Figure 1. Metabolomics (**A,B**). Aldefluour test and development of neural-like phenotype (**C**). qPCR (**D**). CUT&RUN for H3K4me3 (**E**). scRNAseq (**F**).