Lipid treatment induces a neural-like phenotype in non-transformed breast epithelial cells

Mariana Bustamante Eduardo, Shivangi Yadav, Seema Khan, Susan Clare

Department of Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA.

Introduction

- Understanding the genesis of sporadic estrogen receptor (ER-) breast cancer (BC) is a significantly unmet clinical need.
- Previously, we observed that genes involved in **lipid metabolism** are overexpressed in the contralateral unaffected breast (CUBs) of women with ER- BC (1).
- Exposure of non-transformed MCF-10A breast epithelial cells to lipids results in altered chromatin structure, epigenomic reprogramming and changes in gene expression. Within the upregulated genes are numerous gene sets related to nervous system development, neurons and synapses (2).
- Neural genes are more highly expressed in Triple Negative Breast Cancers (TNBC) (3) and the TNBC subtype C2 has high neurogenesis activity (4). Several studies have shown a connection between neural stemness and tumorigenicity (5).

Our aim is to identify potential mechanisms that link the observed lipid-induced expression of neural genes and the genesis of ER- BC.

- We hypothesized that stem-like cells have a survival advantage when exposed to lipids and that lipid-induced molecular changes underlying malignant transformation are connected with a neural-like phenotype.

Methods

- MCF-12A were exposed to vehicle or octanoic acid (OA) for 24 hours. Gene expression was assayed by RNA-seq and OA-responsive genes were identified. Gene set enrichment analysis and the Enrichr online tool were utilized to identify gene sets affected by OA.
- Neural genes identified to be upregulated in TNBC (3) and C2 subtype as well as Neural Crest and Schwann cell markers were compared to OA responsive genes in MCF-10A and MCF-12A.
- The Aldefluour assay was used to identify stem-like (ALDH+) cells in vehicle and OA treated MCF-10A cells.
- To determine if lipid-exposed cells adopt a neural-like phenotype, MCF-10A 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(1):3065. doi: 10.1038/s41598-020-60030-5 (2020).

4. Jézéquel, P. *et al.* Identification of three subtypes of triple-negative breast cancer with potential therapeutic implications. Breast Cancer Res. 2019 May 17;21(1):65. doi: 10.1186/s13058-019-1148-6. PMID: 31101122; PMCID: PMC6525459.

5. Cao Y. Neural is Fundamental: Neural Stemness as the Ground State of Cell Tumorigenicity and Differentiation Potential. Stem Cell Rev Rep. 18(1):37-55. doi: 10.1007/s12015-021-10275-y (2022).

Lipid Exposure

Upregulation of neural-related genes

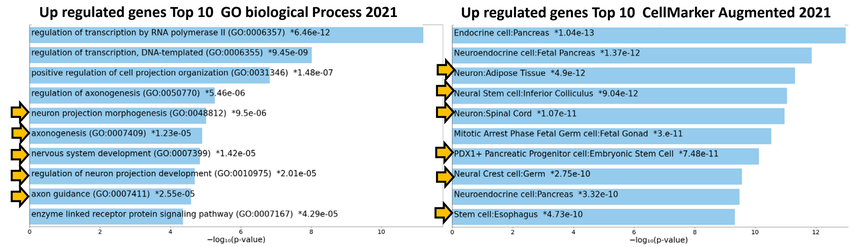
Development of neural-like phenotype

Enrichment of ALDH+ cells

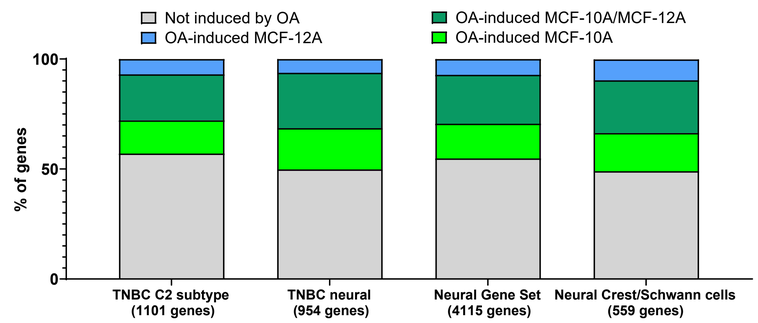
This suggests that lipid exposure results in cell state instability or plasticity, leading to reprogramming/selecting cells with a multi-potential embryonic or stem-like state that has been associated with tumor progression.

Results

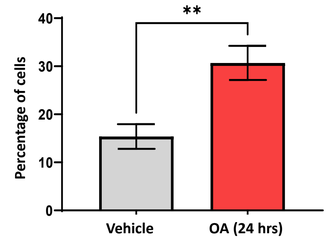
A OA-modulated genes were enriched in Neural- and Stem-related gene sets



B OA induces the expression of neural genes including those upregulated in TNBC, C2 subtype genes and Neural crest/Schwann cells' genes



C ALDH+ cells are enriched upon OA



D Cells develop a neuronal-like phenotype upon OA

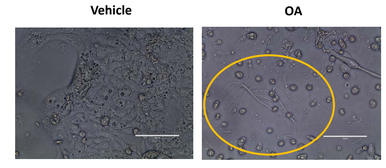


Figure 1. OA treatment is associated with the expression of neuronal-related genes (A-B); the enrichment of ALDH+ cells (C), and the development of neural-like phenotype (D).

mariana.bustamante@northwestern.edu