Elucidating the role of fatty acid metabolism in the genesis of estrogen receptor negative breast cancer

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) has been a persistent focus of our research group. Analysis of gene expression in the epithelial cells from the contralateral unaffected breasts of BC patients identified a lipid metabolism gene signature enriched in women with ER- BC (1). To study this association, we have developed an *in vitro* system to study the effects of fatty acids (FA) on non-transformed breast epithelial cells. The effects of FA were extensive (Figure 1). Yet, the mechanisms by which FA induce these molecular changes remain to be elucidated.

Figure 1. Lipid induced molecular changes (2).

We aim to identify the mechanism by which FA induces molecular changes that potentially promote malignant transformation.

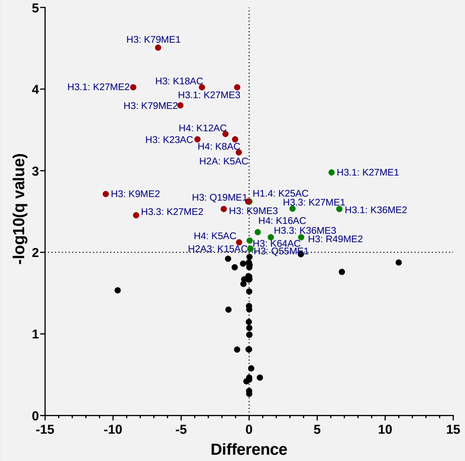
We hypothesized that the increased flux through the ETC controls the modulation of epigenetics.

Methods

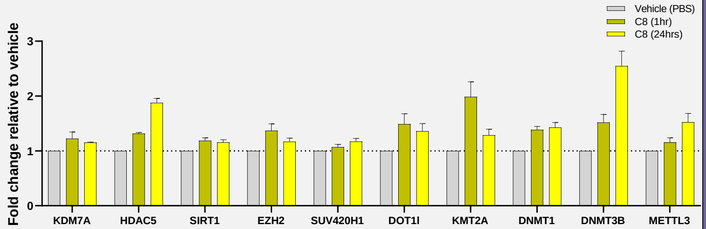
- MCF10A cells were expose to octanoic acid (C8) or linoleic acid (C18) in presence or absence of Complex III (Antimycin A) and Complex I (Metformin) inhibitors.
- Histone PTM were assessed using an Epiproteomic Histone Modification Panel.
- Gene expression was assessed by rt-qPCR.

Results

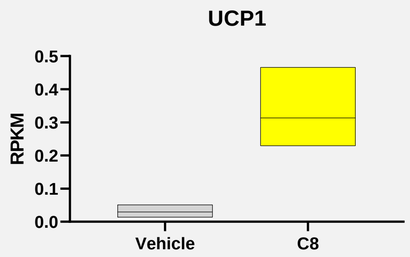
A Methylation and acetylation change significantly following 24 hours exposure to C18.



B Exposure to C8 for 1 and 24 hours alters modulation of epigenetic modulators



C Uncoupling protien 1 expression is increased 3.3X by exposure to C8 for 24 hours



D C8 exposure and inhibition of the ETC modulate gene expression in the same direction

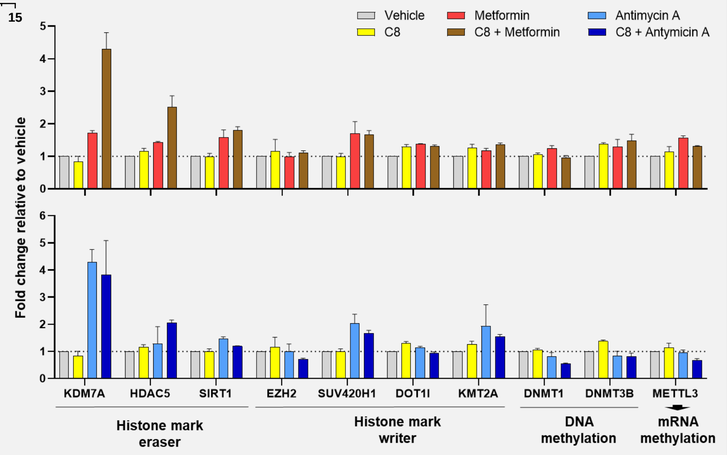


Figure 2. A, Volcano plot of multiple unpaired t tests of histone marks. Relative abundance significantly increased (green) and decreased (red) changed after C18 exposure. **B**, mRNA analysis by RT-qPCR of epigenetic players after 1 hour or 24 hours C8 treatment. **C**, RNA-seq expression analysis of uncoupling protein 1 (UCP1) after C8 treatment (2). **D**, mRNA analysis by RT-qPCR of epigenetic players C8 treatment in presence or absence of ETC inhibitors. Error bars represent the SEM.

Conclusions

- FA altered histone PTMs and the expression of epigenetic modifiers.
- ETC inhibition and lipids affect gene expression similarly, that is, in the same direction. The modulation of epigenetic effectors does not appear to be a consequence of increased flux through the ETC but, rather, a consequence of cellular adaptation to increased flux. Based on this data, we hypothesize that this adaptation occurs through the expression of mitochondrial uncoupling proteins (Figure 3).

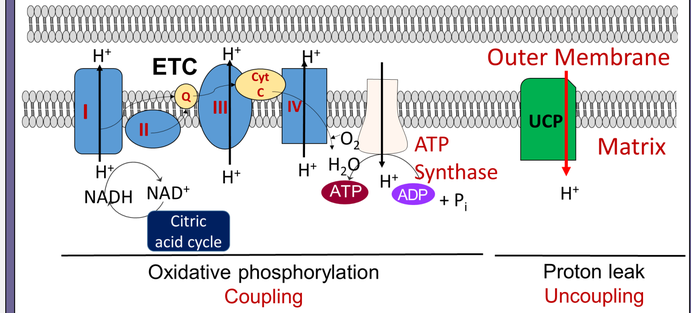


Figure 3. Coupled and uncoupled respiration. Oxidative phosphorylation in mitochondrial electron transport chain (ETC), and proton leak via uncoupling proteins (UCPs).

- We will test our hypothesis by introducing an uncoupler to our model system and also by measuring membrane potential with and without ETC inhibitors.

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.* Exposure of mammary cells to lipid activates gene expression changes associated with ER-negative breast cancer via chromatin remodeling. *bioRxiv*, 2020.2012.2013.422540, doi:10.1101/2020.12.13.422540 (2020).