**Medium Chain Fatty Acids Shift Metabolism Towards the *De Novo* Serine Pathway Fostering Epigenetic Plasticity and Oxidative DNA Damage**

Mariana Bustamante Eduardo1; [Curtis McCloskey](https://pubmed.ncbi.nlm.nih.gov/?sort=date&term=McCloskey+CW&cauthor_id=37883228)2; Gannon Cottone1; Shiyu Liu3; Flavio Palma4, Maria Zappia5; A.B.M.M.K. Islam5; Maxim Frolov5; Elizaveta Benevolenskaya5; Marcelo Bonini4; [Rama Khokha](https://pubmed.ncbi.nlm.nih.gov/?sort=date&term=Khokha+R&cauthor_id=37883228)2; Navdeep Chandel6, Seema Khan1, Susan Clare1

1Department of Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL, USA. 2Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada. 3Department of Pharmacology and Cancer Biology, Duke University, Durham, NC, USA. 4Department of Medicine/Division of Hematology Oncology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA. 5Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL, USA. 6Division of Pulmonary and Critical Care Medicine, Department of Medicine, Northwestern University, Chicago, IL, USA.

Fatty acid (FA) exposure in breast tissue increases flux through the *de novo* serine synthesis pathway (SSP), alters histone methylation and gene expression The association of the SSP and estrogen receptor-negative breast cancer (ERnegBC) was first observed over a decade ago. We hypothesize that FA induces a metabolic shift towards the SSP promoting ERnegBC.

Non-transformed MCF-10A cells were exposed to the medium-chain FA octanoic acid (OA) for U13C-glucose tracing and proteomics. Targeted metabolomics and Western blot analyses were conducted on cells exposed to OA ± SSP inhibitors. ROS-induced redox changes were monitored live, DNA damage was assessed using the comet assay, and CUT&RUN was performed for H3K4me3. Human breast tissue derived microstructures exposed to ± OA were utilized for single-cell RNA-seq analysis.

U13C-glucose tracing in presence of OA revealed that one-carbon-THF was redirected to the methionine cycle increasing flux to methylation. OA increased the SSP enzyme PHGDH, as well as S-adenosylmethionine, glutathione and 2-hydroxyglutarate. Blocking PHGDH, prevented these increases. Blocking the SSP enzymes PHGDH and PSPH blocked the increase expression of OA-induced proteins. The SSP transcription factor *ATF3* and genes *PHGDH* and *PSAT1* increased with OAin microstructures. Basal BSL1, hormone sensing HS1 and luminal progenitor LP3 subtypes showed an increased flux through the SSP. The SSP transcription factors ATF3/4 (p < 0.05) motifs were enriched at H3K4me3 peaks. After 5 min OA exposure, mitochondrial and nuclear ROS increased significantly (p < 0.01), peaking at 15 min. OA exposure triggered DNA damage likely due to ROS increase in the nucleus. OA increased glutathione metabolism and ROS detoxification in BSL1.

PHGDH is elevated in 70% of ERnegBCs, despite its gene being amplified in only 6% of all BCs, implying other mechanisms are involved in PHGDH dysregulation. One possibility involves the FA-induced shift towards the SSP increasing S-adenosylmethionine, 2-hydroxyglutarate and glutathione. This promotes epigenetic phenotypic plasticity and controls ROS, thereby supporting the survival of cells that acquire DNA damage and potentially facilitating carcinogenesis. This understanding opens possibilities for novel preventive strategies. For instance, targeting the first or third enzyme of the SSP could block the oncogenic effects induced by this metabolic shift.