RNAi Screening in Primary Human Breast Biopsies Identifies Mitochondrial Glutamate Flux as A Metabolic Vulnerability in Locally Advanced Breast Cancer

Caterina Iorio
Sunnybrook Research Institute
Alla Bouzina
Sunnybrook Research Institute
Katarzyna Jerzak
Sunnybrook Health Sciences Centre
David Andrews
Sunnybrook Research Institute
Robert Screaton (robert.screaton@sri.utoronto.ca)
Sunnybrook Research Institute https://orcid.org/0000-0002-4917-9473

Research article

Keywords: SLC25, mitochondria, breast cancer, high-throughput screening (HTS), locally advanced breast cancer, RNA interference (RNAi), primary human cells

Posted Date: March 3rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-260480/v1

Abstract

Background: Breast cancer (BC) is a leading cause of death in women[1]. Women with Locally Advanced Breast Cancer (LABC) have high risk disease with either large primary breast tumours and/or lymph node involvement. While neoadjuvant chemotherapy eradicates breast cancer in approximately one-third of cases prior to surgery, almost 70% of patients have residual disease and many will require additional chemotherapy post-operatively. Improving pre-operative efficacy of neoadjuvant systemic treatments while reducing their iatrogenicities are critical unmet needs.

Methods: Here, we develop an RNA interference (RNAi) screening approach using conditionally reprogrammed primary LABC biopsies to identify genes of the mitochondrial Solute Ligand Carrier 25 (SLC25) family that support LABC cell viability.

Results: We report that silencing SLC25A12, -A15, and -A18 genes, involved in glutamate and ornithine flux, augment 5-fluorouracil (5FU) cytotoxic effectiveness in LABC cells.

Conclusions: Our data suggest glutamate metabolism may be a tumour-specific metabolic vulnerability in LABC. Furthermore, we demonstrate that RNAi screening in conditionally reprogrammed primary human breast cells can identify novel targets for the development of non-genotoxic BC treatments.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and accessed as a PDF.

Figures

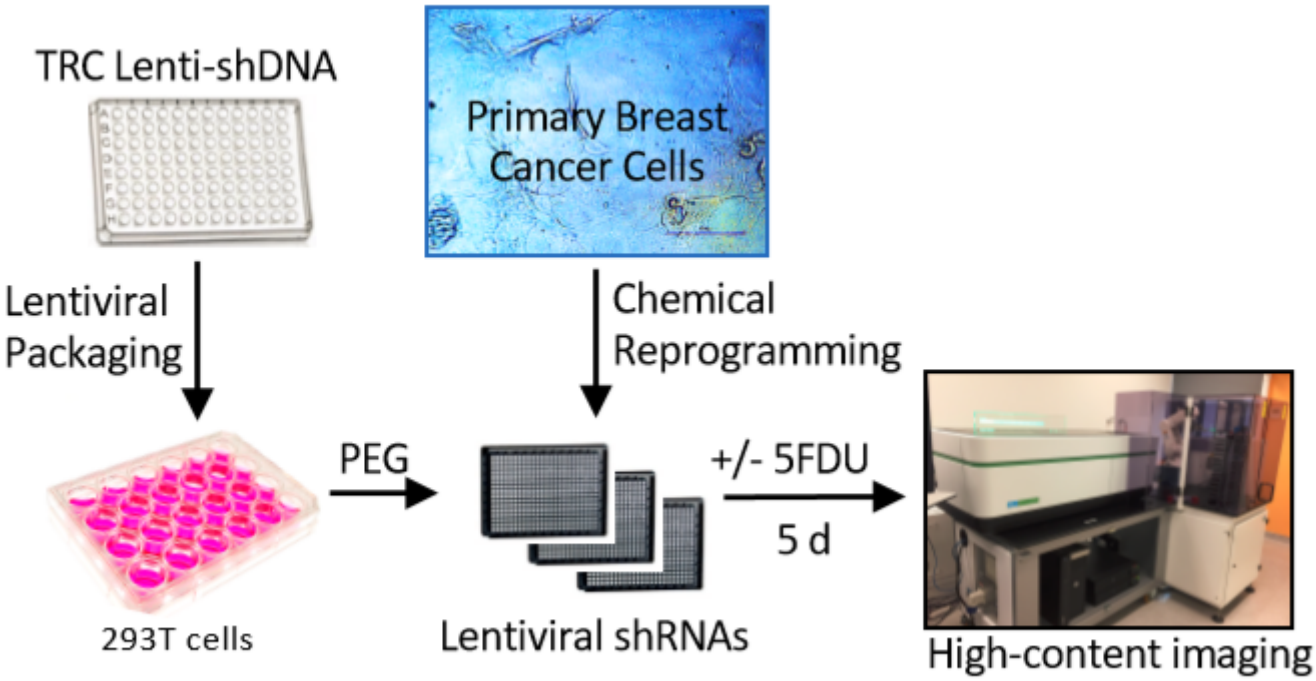

Figure 1

HTS lentiviral screening in primary human breast tumours. Lenti-shDNAplasmids from SIGMA TRC1, 1.5 and 2 libraries are transfected together with lentiviral genome plasmids in HEK293T cells to package lentiviral-shRNAs. Lentivirus are arrayed in 384 well plates and cultured with LABC cells (or control adenoma cells) with or without a low dose of 5FDU.

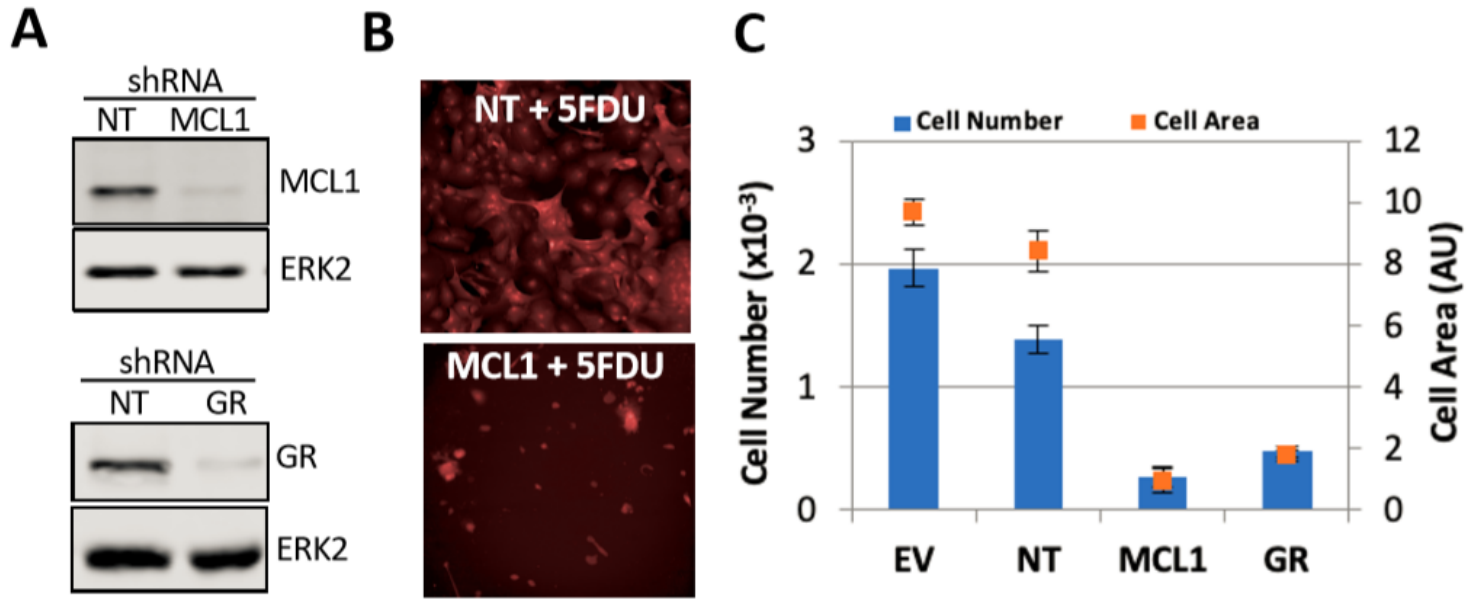

Figure 2

Synthetic lethal screen. A. Western blots showing silencing of the positive controls for screen, prosurvival protein MCL1 and the glucocorticoid receptor, GR.B. Photomicrographs of images of LABC1 cells expressing nontargeting (NT) or MCL1 control shRNA treated with an EC20 of 5FDU. C. Barplot showing cell (nuclear) number (blue bars) and cell area (orange squares) for the indicated shRNAs in the presence of EC20 5FDU. EV = Empty Vector. NT = nontargeting control.

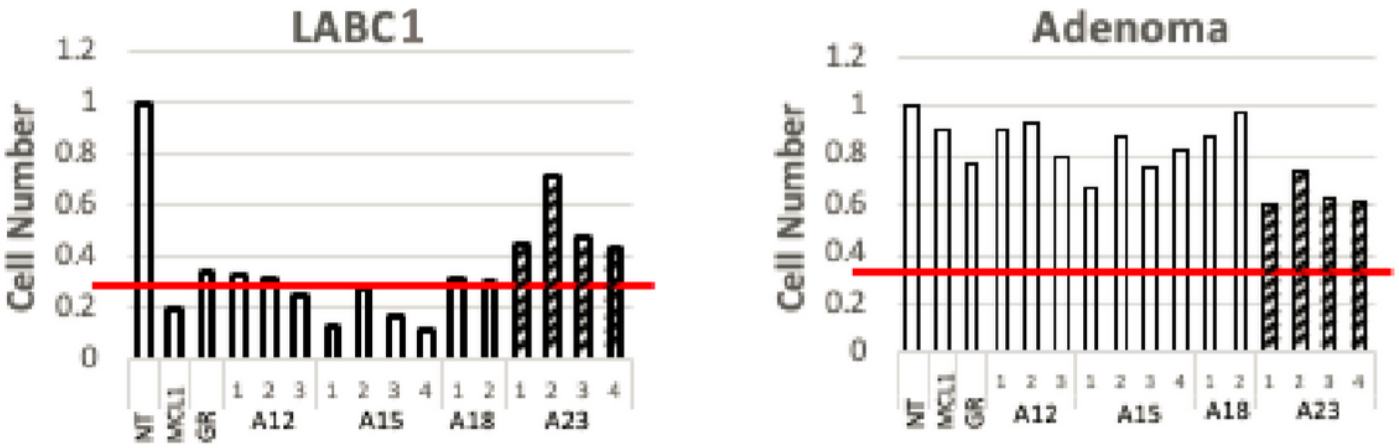

Figure 3

SLC25 mitochondrial carrierscreen results. In the presence of an EC20 of 5FDU, only LABC1 cells (left) die following silencing of mitochondrial carriers for glutamate (A12, A18) and ornithine (A15). Adenoma cells (right) are unaffected. Red line = threshold established by cell death observed in positive controls MCL1 and GR. Hashed bars indicate partial death sensitization effect of the ATP-Mg carrier A23 in both cell types.