Notch Dependency in Triple-Negative Breast Cancer Mitochondrial Metabolism

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Introduction

Triple Negative Breast Cancer (TNBC) makes up 15 – 20% of breast cancers. It has the worst prognosis amongst the breast cancer carcinomas. TNBC lacks expression of human epidermal growth factor receptor 2 (HER2) and the endocrine receptors for estrogen and progesterone. Because it is negative for all three therapeutic targets, TNBC is difficult to treat. Chemotherapy is the standard for treatment of TNBC, however, about 80% of TNBC patients do not completely respond to chemotherapy. Resistance to therapy and the recurrence of breast cancer is thought to be caused by breast cancer stem cells. Notch signaling (canonical) is an identified contributor to breast cancer and cancer stem cell maintenance. Recent studies also highlight the importance of non-canonical Notch signaling. Previously, we reported that Notch signaling regulates mitochondrial metabolism in TNBC and is present on the mitochondrial membrane. *However, the role of non-canonical Notch signaling in TNBC metabolism is unknown.* This study aims to determine the role of non-canonical Notch signaling in TNBC mitochondrial metabolism.

We analyzed the metabolic profile of MDA-MB-231 human TNBC cells and mammospheres using Seahorse Analyzer. Mammospheres were generated using Mammocult medium (STEMCELL Technologies) as per manufacturer protocol. The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were assessed for Notch1 over-expressed cells (N1IC) and mammospheres versus the vectors (controls). Afterward, sulindac sulfide (SS), (10μM) a non-steroidal anti-inflammatory drug was used as a Notch inhibitor.

Notch1 co-localizes with Mitochondria in TNBC

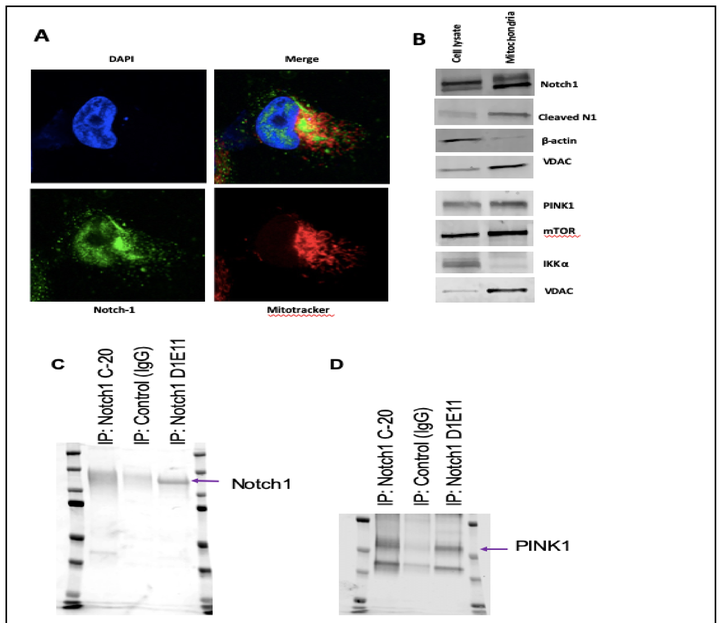

Figure 1. Notch1 co-localizes with Mitochondria in TNBC cells. **(A)** MDA-MB-231 cells were visualized by confocal microscopy after being stained with mitochondrial tracer (MitoTracker), Notch1 (C-20) antibody, and DAPI (nucleus). **(B)** Mitochondria were isolated using Mitochondria Isolation Kit (Abcam) and Western blot was used to determine protein expression [A & B (PMID: 30564555]. **(C)** Mitochondrial Notch1 immunoprecipitation from MDA-MB-231 cells by two different antibody fractions [Cell signaling (D1E11) and Santa Cruz Biotechnology (C-20)] and western blot analysis. **(D)** Detection of PINK1 by western blotting.

MDA-MB-231 Mammospheres have increased oxidative metabolism

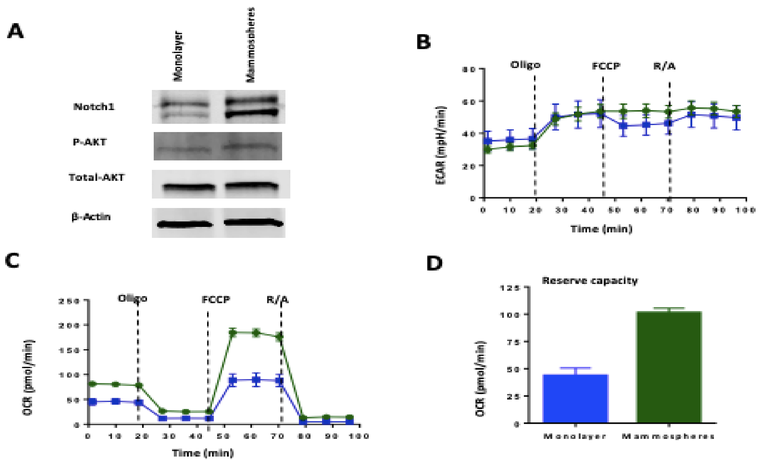

Figure 2. (A) Expression of Notch1 and AKT phosphorylation was determined by Western blotting. **(B)** ECAR and **(C)** OCR including **(D)** reserve capacity were measured by Seahorse Analyzer (PMID: 30564555).

Notch1-IC (N1IC) cells have increased metabolic activities

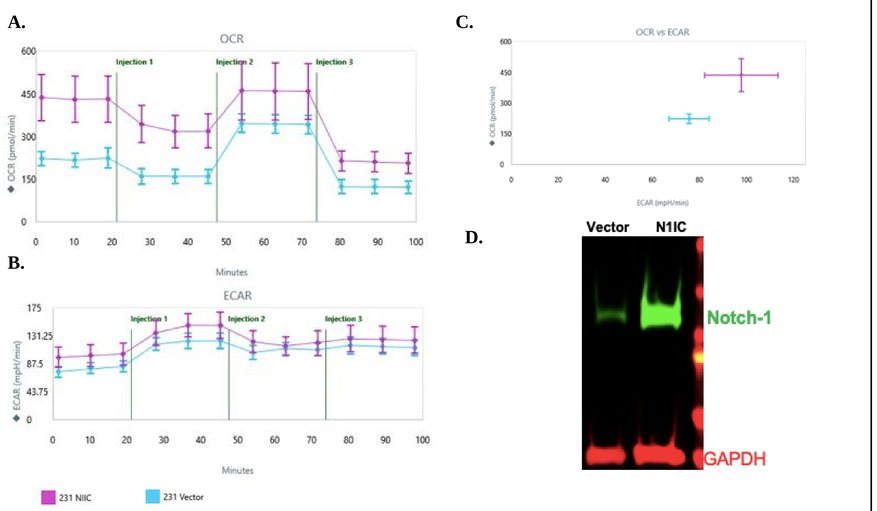

Figure 3. Metabolism of N1IC MDA-MB-231 cells compared to MDA-MB-231 vector control cells using Seahorse Analyzer. **(A)** OCR metabolism **(B)** ECAR metabolism **(C)** OCAR vs ECAR metabolism **(D)** Confirmation of Notch1 overexpression

Notch1-IC (N1IC) Mammospheres have increased metabolic activities

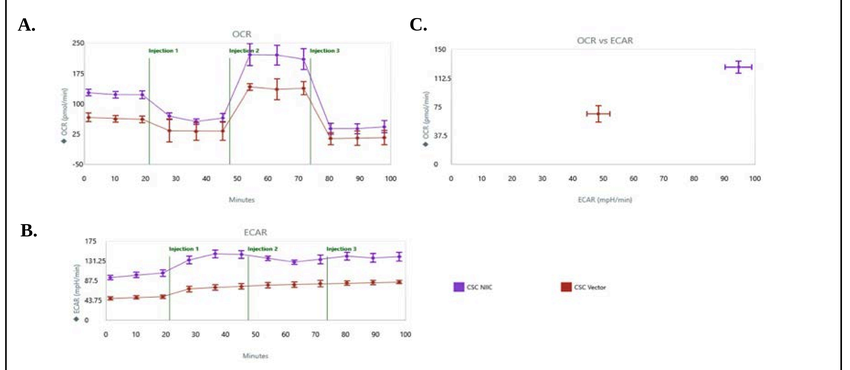

Figure 4. Metabolism of mammosphere N1IC Cancer Stem Cells (CSC) compared to CSC vector control using Seahorse Analyzer. **(A)** OCR metabolism **(B)** ECAR **(C)** OCAR vs ECAR metabolism

SS mediated anti-mammosphere metabolism depends on Notch expression

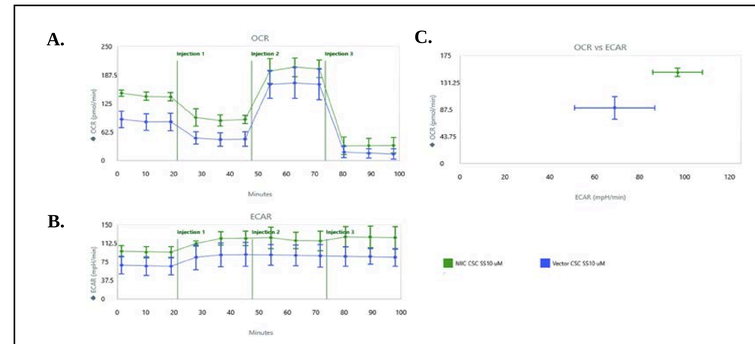

Figure 5. Seahorse Analyzer compared metabolism of mammosphere N1IC Cancer Stem Cells (CSC) to CSC vector control after administering sulindac sulfide, (10μM). **(A)** OCR metabolism **(B)** ECAR metabolism **(C)** OCAR vs ECAR metabolism

Conclusion and Future Directions

- **Notch1 overexpression increased cellular metabolic activities**
- **Our results suggest that the mitochondrial metabolic activity is Notch dependent.**
- **Our next step is to run proteomics on the samples to determine the associated mitochondrial proteins that are also influenced by non-canonical Notch signaling. Our lab received an IDeA National Resource for Quantitative Proteomics Voucher from the University of Arkansas for Medical Sciences.**
- **This will help point us towards the direction of possible therapeutic targets for TNBC treatment.**

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