WNT4 regulates cellular metabolism via intracellular activity at the mitochondria in invasive lobular carcinoma cells

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Lay Abstract

Proteins called 'Wnt ligands' are important for how tissues and organs in the body grow and develop, but we found that one Wnt ligand important in invasive lobular carcinoma (ILC) - WNT4 - has unusual functions in these cancer cells. In ILC, WNT4 is important for controlling how cancer cells metabolize fuels to support their growth and survival, and may direct ILC cells to preferentially use fats and amino acids instead of sugars. This parallels some observations on how ILC tumors use fuel. To learn about WNT4 in ILC metabolism, we used mass spectrometry to identify cellular proteins that WNT4 may partner with. These studies showed that, unexpectedly, WNT4 is physically located at the mitochondria, the sub-cellular units that produce energy from available fuels. This work will help us understand how ILC use fuel, and help identify treatment approaches targeted against the specific ways that ILC metabolize fats, proteins, and sugars.

Scientific Abstract

Wnt ligand WNT4 behaves in an atypical manner in invasive lobular carcinoma (ILC) and is a key ILC-specific estrogen receptor (ER) target. We previously showed that WNT4 engages with the PI3K-mTOR pathway via p70S6K and maintains downstream pathway activation, ultimately regulating cellular proliferation, metabolism, and survival. In ILC cells, we found WNT4 is critical in mitochondrial function and oxidative phosphorylation (OXPHOS), but the specific mechanisms are not yet understood. To explore the mechanistic role for WNT4 in ILC metabolism, we performed proximity-dependent biotinylation with mass spectrometry (BioID) to identify WNT4 signaling partners. BioID showed that unlike canonical Wnt protein WNT3A, WNT4 was localized to the mitochondria with putative partners STAT1, DHRS2, and mTOR. We explored WNT4 regulation of cellular metabolism further using global metabolomics comparing the effects of WNT4 knockdown inhibition or over-expression. We observed WNT4 signaling regulates OXPHOS; WNT4 knockdown suppressed OXPHOS as well as fatty acid and glutamate metabolism pathways, but not glycolytic activity. Taken together these data support that WNT4 influences cellular metabolism and mitochondrial function and plays a key role in unique metabolic phenotype of ILC. Determining the role of WNT4 within the mitochondria will allow us to better understand ILC metabolism and therefore how to better target WNT4 signaling pathways for potential treatment options.

WNT4 Behaves in Atypical Manner Downstream ERα

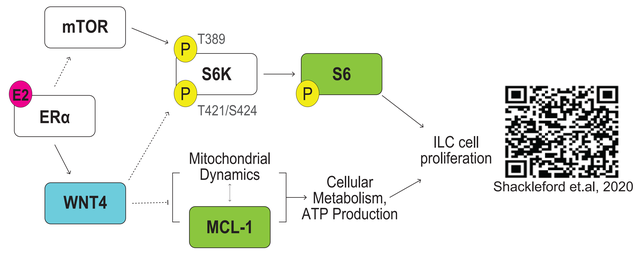

Fig 1. Previously observed WNT4 downstream interaction with MCL-1.

BioID Intracellular Protein Association Stable Lines

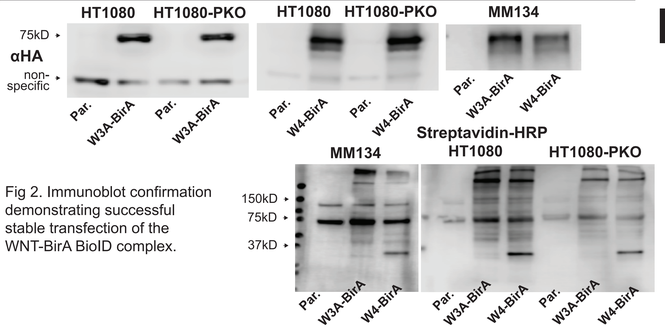

Fig 2. Immunoblot confirmation demonstrating successful stable transfection of the WNT-BirA BioID complex.

WNT4 Localizes to the Mitochondria Instead of Trafficking for Secretion

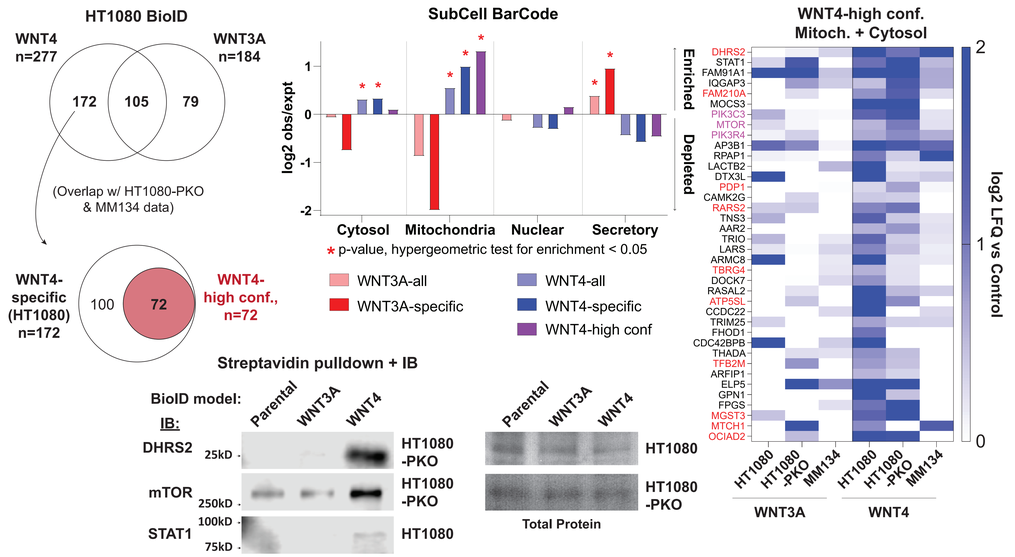

Fig 3. Analysis of intracellular proteins biotinylated via proximity to WNT-BirA.

Metabolites Dysregulated via WNT4 Knockdown Mirror ER Knockdown

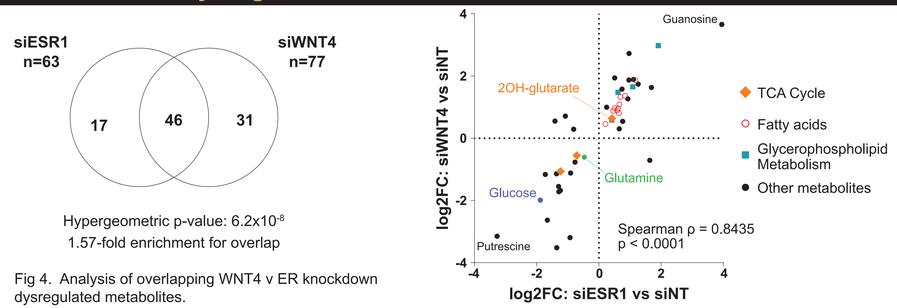

Fig 4. Analysis of overlapping WNT4 v ER knockdown dysregulated metabolites.

WNT4 Knockdown Dysregulates OXPHOS but not Glycolysis

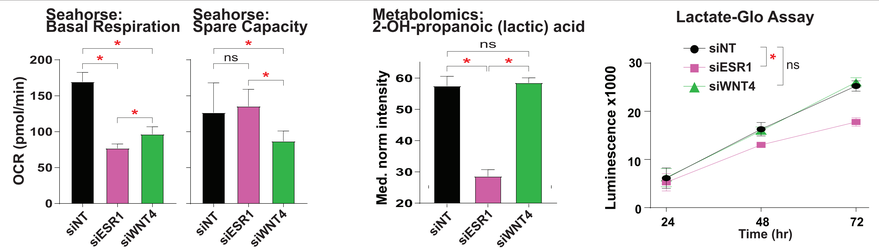

Fig 5. Analysis of WNT4 v ER knockdown impact on cellular respiration and lactic acid levels in ILC.

WNT4 is Involved in Fatty Acid Metabolism

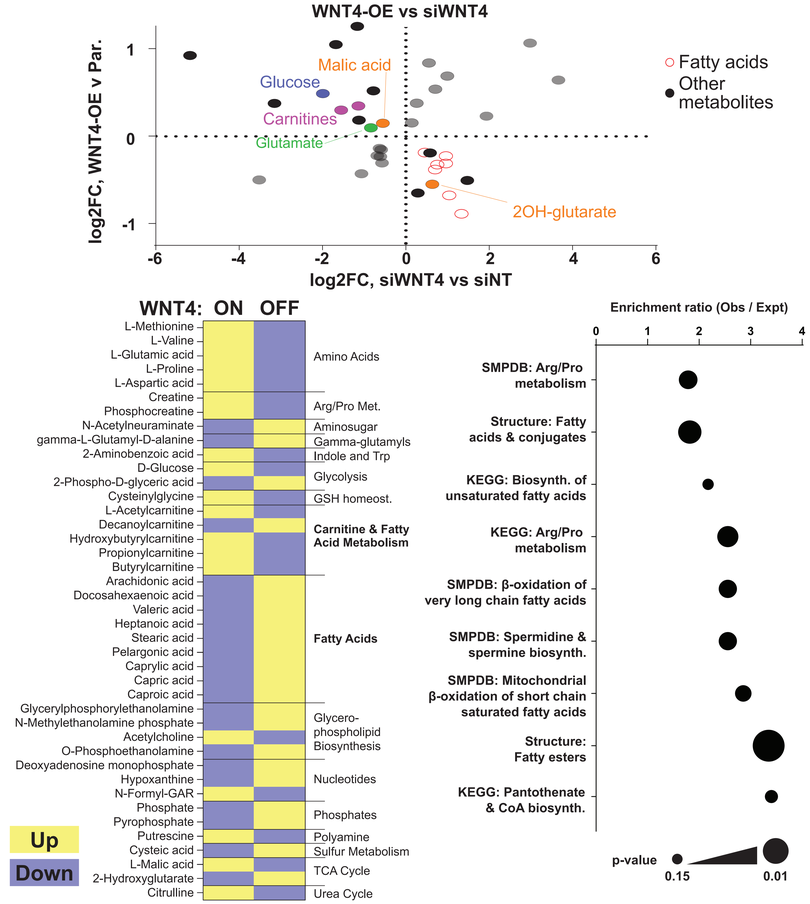

Fig 6. Identification of metabolites and metabolic pathways regulated by WNT4.

Conclusions

- WNT4 is localized to the mitochondria as demonstrated using BioID
- WNT4 signaling is necessary for cellular respiration in ILC
- It is likely that WNT4-mediated metabolic regulation is independent of glycolytic activity, pointing toward ILC cells being more reliant on OXPHOS, fatty acid and glutamate metabolic pathways.

Future Directions

- Explore the location and role WNT4 plays within the mitochondria.
- Further specify where within identified metabolic pathways WNT4 interaction is occurring.
- Use the above to better target WNT4 signaling pathways for better treatment options.

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