Resolving Subcellular Metabolic Flux

Evan Quon[1], Lucas Sullivan[1]

Human Biology Divisions, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

equon@fredhutch.org
@quon_evan
Hutchinson C3-187

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Introduction

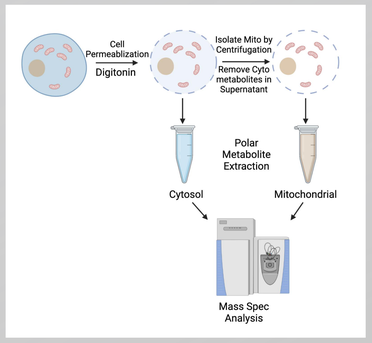
Subcellular compartmentalization is an intrinsic feature of eukaryotic cells. Each type of compartment has a distinct cellular function that requires a specific metabolic environment. Maintaining these functionally and metabolically distinct regions within the cell allows for metabolic flexibility and the ability to respond to stress. Considering that altered cellular metabolism is a hallmark of cancer, there is an imperative need to understand which compartment specific alterations are necessary for cancer cells to survive which can be leveraged into developing anticancer therapies. By deconvolving the metabolic alterations specific to the cytosol and the major metabolic hub of the cell, the mitochondria, we may be able to discover compartment specific alterations which would otherwise go undiscovered by assaying whole cell metabolite extractions. Therefore, we adapted a subcellular fractionation method that rapidly enriches for mitochondria, preserving the metabolic state and allowing for mitochondrial metabolomics analysis compared to whole cells, whose signal is dominated by cytosol.

Objective

Develop and assay that specifically and rapidly enriches for mitochondrial metabolites to deconvolve mitochondrial and cytosolic specific metabolic networks

Methods

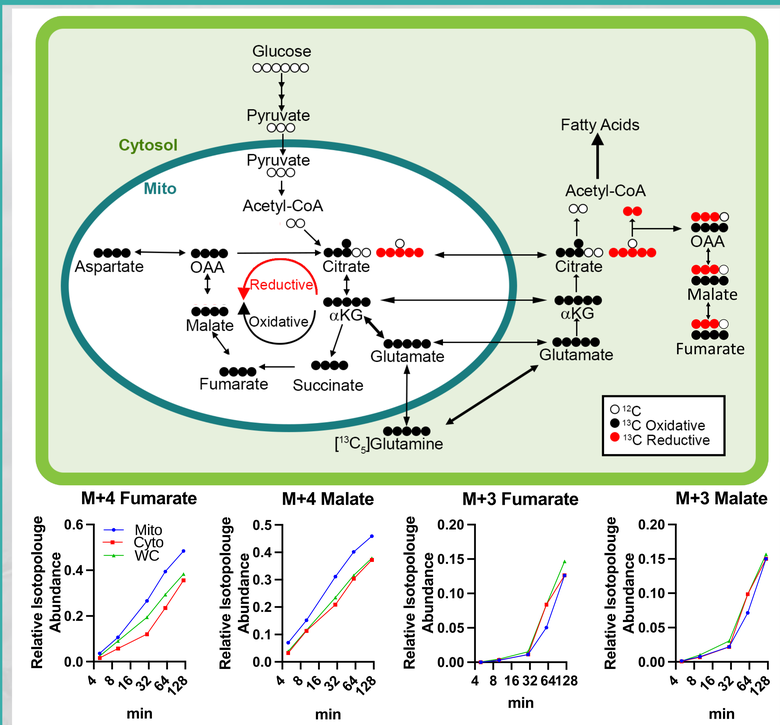
1. Treat cells with drug and stable isotope label
2. Permeabilize PM with Digitonin
3. Separate Cytosol (Supernatant) from mitochondria (Pellet)
4. Extract metabolites
5. LC-MS Analysis



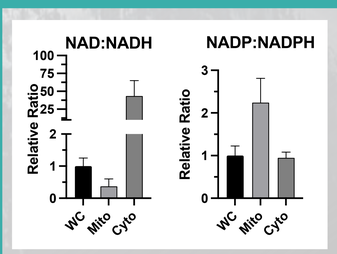
Protocol Adapted from Lee et al (2019) Nature Communications

Results

1. Oxidative and Reductive TCA Metabolism Are Compartment Specific



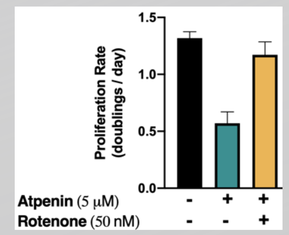
2. Mito and Cyto have distinct cofactor redox states



Question

How does disrupting mitochondrial metabolism distinctly affect the metabolic circuitry and redox state of the mitochondrial and cytosolic compartments?

3. SDHB inhibition by Atpenin A5 treatment causes proliferation defects that are partially rescued by Complex I inhibition (Rotenone)



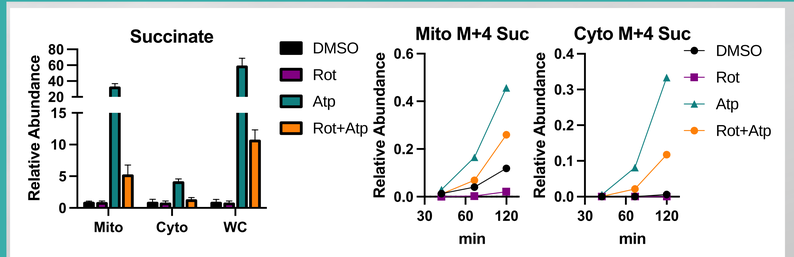
Madeleine Hart

mlhart@fredhutch.org

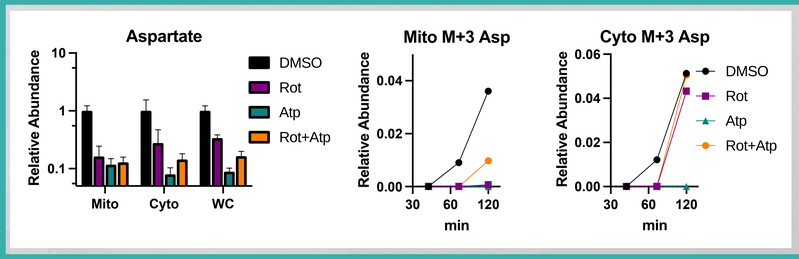
@madelouhart

Check out her poster to find out more about the consequences of SDHB inhibition!

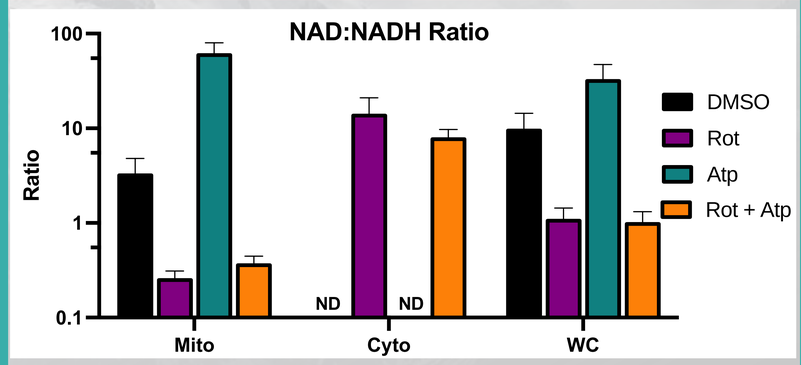
4. SDHB inhibition by Atpenin A5 treatment causes whole cell succinate accumulation which is repressed by Complex I inhibition



5. Complex I inhibition partially rescues aspartate depletion in the Atpenin A5 treated cells by supporting Cytosolic Reductive TCA cycling



6. Rotenone treatment restores redox homeostasis in Atpenin A5 treated cells which may facilitate oxidative TCA metabolism



Future Directions and Applications

- Determine the compartment specific adaptations to various mitochondrial metabolic inhibitions (ie metformin)
- Determine the compartment specific NAD(P)/H ratios and how they collectively and individually contribute to cellular redox homeostasis
- Explore the subcellular effects of metabolic driven tumors
- Expand this protocol to rapidly isolate other subcellular compartments (ie nucleus)