MEDICAL COLLEGE OF WISCONSIN CANCER CENTER

Bioenergetics Shared Resource

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OVERVIEW

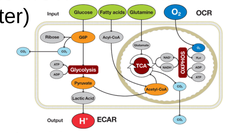
The MCW Cancer Center Bioenergetics Shared Resource opened in January 2012 and became fully operational in February 2012. The facility is located in the Department of Biophysics in MFRC 2013.

The purpose of the Bioenergetics Shared Resource is four-fold:

- investigate cancer cell metabolism and understand how cancer cells exploit metabolic pathways for survival,
- provide a better understanding of the bioenergetic pathways in cancer metabolism during hypoxia and normoxia,
- assess new metabolism-based strategies for cancer treatment, and
- promote increased collaboration in cancer research between basic scientists and clinical researchers.

SERVICES PROVIDED

- Metabolite analysis
- Assessment of mitochondrial and glycolytic function
- Longitudinal studies in tumorigenesis models
- Mitochondrial toxicity of compounds
- Measure ROS (in association with the MCW EPR Center and the Free Radical Research Center)



Research topics:

- Synergistic effects of metabolic Inhibition of breast cancer
- Identification of epithelial progression to skin cancer cells
- Identification of breast cancer cell biochemical pathways and mitochondrial function
- Synergistic antitumor inhibitory effect of 3-BRPA in combination with mTOR inhibitor rapamycin *in vivo* and *in vitro*
- Metabolic and bioenergetic effects of natural compounds in cancer cells
- Imaging of ^{13}C glycolytic and mitochondrial metabolites *in vitro and in vivo*

STRUCTURE OF ACCESSIBILITY

Balaraman Kalyanaraman, Ph.D. – Director, Bioenergetics Shared Resource
• *Expert in mitochondrial ROS, cancer, and Parkinson's disease*

Jacek Zielonka, Ph.D. – Research Scientist II
• *Expert in ROS/Mass Spectrometry/HPLC*

Michael Mouradian, Ph.D. – Post Doctoral Fellow
• *Expert in cancer metabolism and bioenergetics*
• *Conducts consultations and experiments for the Shimadzu 8030 LC MS/MS*

Steve Komas – Lab Manager/Technologist
• *Conducts all consultations and experiments for the Seahorse XF96*
• *Schedules all experiments for Shared Resource*

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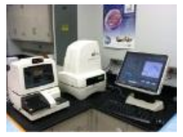
KEY EQUIPMENT

1. Seahorse Bioscience XF96 Extracellular Flux Analyzer



Capabilities:

- Measure mitochondrial function via oxygen consumption
- Measure glycolytic function via change in pH
- Measure fatty acid oxidation
- Add mitochondrial stressors such as oligomycin, FCCP, and antimycin A during the run
- Measure mitochondrial function of cancer cell treatments

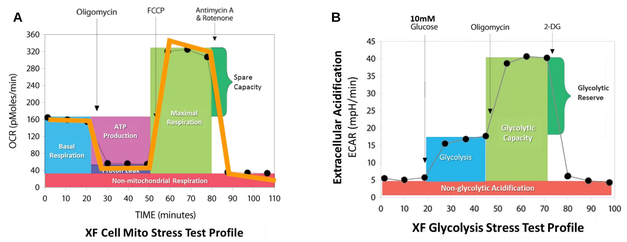


Figure 1: Bioenergetic profiles of Seahorse XF96 extracellular flux analyzer examining A) mitochondrial and B) glycolytic function

Advantages compared to alternatives:

- Capable of running 96-well microplate in a high throughput format
- Decreased sample size compared to Clark electrode
- Capable of simultaneously measuring both mitochondrial function (Figure 1A) and glycolytic function (Figure 1B) via monitoring of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR)
- Enables user to have the capability of measuring mitochondrial function with the addition of mitochondrial inhibitors and protonophores during the experiment (Figure 1A)
- The assay is completely automated

2. Shimadzu Ultra-High Performance Liquid Chromatography / Mass Spectrometry 8030 System

Capabilities:

- Measure metabolites pre-optimized on the instrument
- Measure intracellular uptake of cancer cell treatments
- Measure global metabolic profile and ^{13}C-tracer-based metabolic fluxes

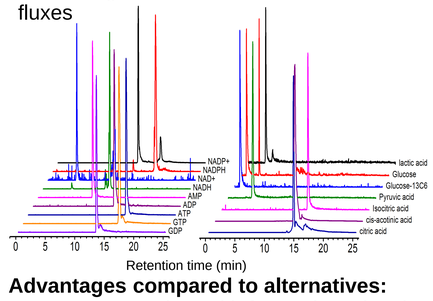


Figure 2: Liquid chromatography retention time of common cellular metabolites as detected by mass spectrometry. 10 µM of each compound was prepared into mobile phase and eluted on a Phenomenex C18 column. Specific daughter ion fragmentation patterns were used to identify each metabolite.

Advantages compared to alternatives:

- Decreases run time with faster detection times
- Capable of measure metabolites in under 25 minutes
- Capable of screening numerous cellular metabolic pathways simultaneously (TCA, glycolysis)
- Completely automated once samples are loaded
- Tandem mass spec breaks parent ion into daughter ions for optimum identification of compounds and metabolites compared to other mass specs

RESEARCH SUPPORTED

Example 1: Bioenergetic analysis of combining mitochondria-targeted drugs with 2-deoxyglucose in breast cancer cells

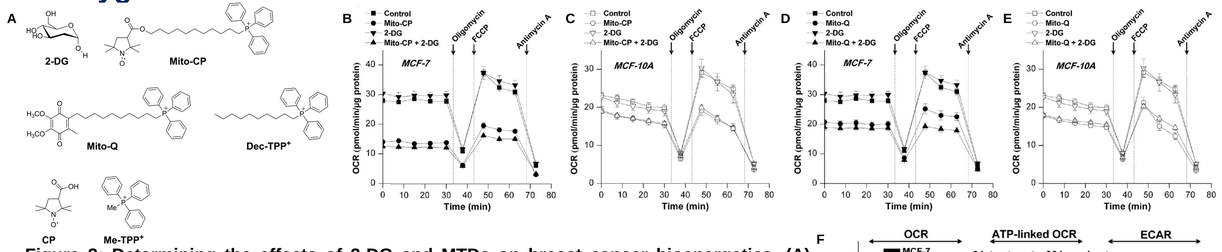


Figure 3: Determining the effects of 2-DG and MTDs on breast cancer bioenergetics. (A) Structures of 2-DG, Mito-CP, Mito-Q, Dec-TPP+, Carboxy proxyl (CP), Me-TPP+. **(B-E)** MCF-7 and MCF-10A cells (20,000 cells per well) were treated with the indicated compounds for 6 hours. The cells were washed with complete media and returned to a 37 C incubator for 36 hours. Five baseline measurements were taken before injection of oligomycin (1µg/mL) to inhibit ATP synthase, FCCP (1-3 µM) to uncouple the mitochondria and yield maximal OCR, and antimycin A (10 µM) to inhibit complex III and mitochondrial oxygen consumption. **(F)** The effects of MTDs and 2-DG on basal OCR, ATP-linked OCR and ECAR. *, P<0.01 (n=5) comparing MCF-7 with MCF10A under the same treatment conditions. [*Cheng G et al. Cancer Res. 2012. 72(10): 2634-44].*

Example 2: Effects of Mito-ChM on basal OCR and bioenergetic function in MCF-7 and MCF-10A cells

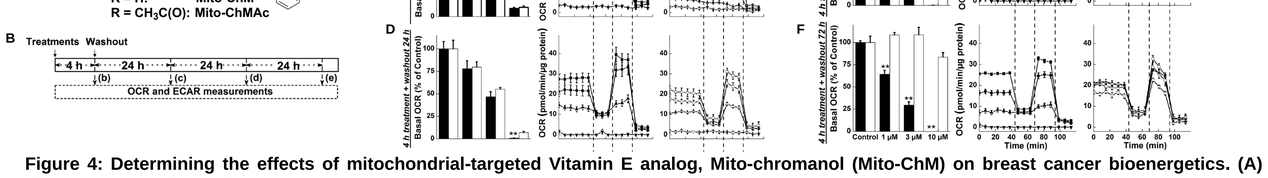


Figure 4: Determining the effects of mitochondrial-targeted Vitamin E analog, Mito-chromanol (Mito-ChM) on breast cancer bioenergetics. (A) Structure of Mito-ChM. **(B)** Experimental protocol for functional assay. **(C)** MCF-7 and MCF-10A cells were assayed for OCR immediately after treatment with Mito-ChM (1-10 µM) for 4 hours, **(D)** after incubation without Mito-ChM for an additional 24 h, **(E)** after additional incubation without Mito-ChM for 48 hours, and **(F)** after additional incubation without Mito-ChM for 72 hours. [*Cheng G et al. BMC Cancer. 2013.13(1):285*].

Example 3: 2D Map of bioenergetics in PDAC – susceptibility to glycolytic inhibitors

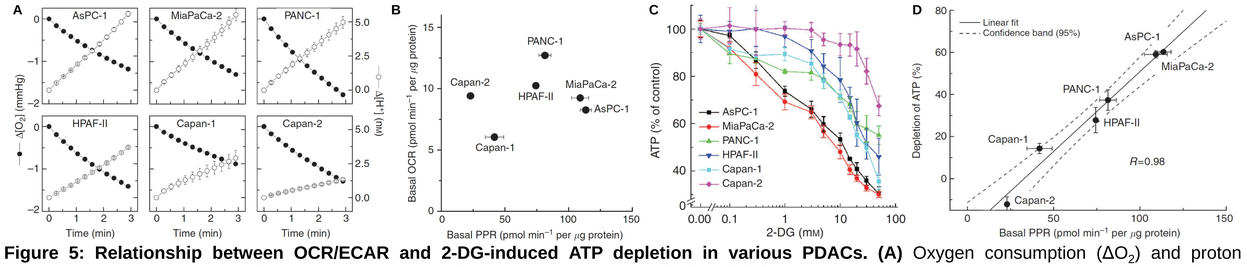


Figure 5: Relationship between OCR/ECAR and 2-DG-induced ATP depletion in various PDACs. (A) Oxygen consumption (ΔO_2) and proton production (ΔH^+) after normalization to 1 µg of protein. **(B)** 2-D map of OCR and ECAR in *PDAC cell lines*. **(C)** Intracellular ATP levels in specified cell lines treated with 2-DG as indicated for 24 hours. **(D)** Relationship between basal ECAR value and 2-DG induced ATP loss (normalized to protein). Values are mean±SD (n=4-6). [*Cheng G et al. Br J Cancer. 2014. May:4-6*].

Publications Using the CCBSR

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3. Starenki D, Park J-I: Mitochondria-targeted nitroxide, Mito-CP, suppresses medullary thyroid carcinoma cell survival in vitro and in vivo. *J Clin Endocrinol Metab* 2013, 98:1529–40.
4. Cheng G, Zielonka J, McAllister DM, Mackinnon a C, Joseph J, Dwinell MB, Kalyanaraman B: Mitochondria-targeted vitamin E analogs inhibit breast cancer cell energy metabolism and promote cell death. *BMC Cancer* 2013, 13:285.
5. Dranka BP, Gifford A, Ghosh A, Zielonka J, Joseph J, Kanthasamy AG, Kalyanaraman B: Diapocynin prevents early Parkinson's disease symptoms in the leucine-rich repeat kinase 2 (LRRK2R^{1441}G) transgenic mouse. *Neurosci Lett* 2013, 549:57–62.
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