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 MFRIC 2013. The purpose of the Bioenergetics Shared Resource is fourfold:

- 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,
- Promote increased collaboration in cancer research between basic scientists and clinical investigators.

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
- Role of epithelial progression to skin cancer cells
- Identification of breast cancer cell biochemical pathways and mitochondrial function
- Mitochondrial anti-apoptotic effect of 3-BPFA in combination with mTOR inhibitor rapamycin

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) by 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 protonsopholes 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 cells treatments
- Measure global metabolic profile and 13C-tracer-based metabolic fluxes

Figure 2: Liquid chromatography retention time of common cellular metabolites as detected by mass spectrometry. 10 μl 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 spec analyses.

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) Structure of 2-DG, Mito-OP, Mito-Q, Dec-TPP+, Carbonyl prox (CP), Mito-TPP+, (B) 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 OCR values were taken beginning with exposure 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 cytochrome bc1 and yield maximal ECAR. (C) Three different cell lines tested of 2-DG on basal OCR, ATP-linked OCR and ECAR, *P<0.01 (n=5) comparing MCF-7 with MCF-10A 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 and 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. [Cheng G et al. BMC Cancer 2013, 13(11):2056]

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 PDACAs. (A) Oxygen consumption (ΔOCR) and proton production (ΔATP) 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 means/SD (n=4). [Cheng G et al. Br J Cancer. 2014, May 6]

PUBLICATIONS USING THE CCSRB


Gaggl T, Mailotier P. Do not fast acidosis/hypoxia contribute significantly to establishment of a bioenergetically favorable environment for vaccine virus infection. PLoS Pathog 2014, 10(10)1004791.


