Characterization of Metabolic Changes Induced by Anticancer Drugs in MCF-7 and MDA-MB-231 Cell Lines

2568

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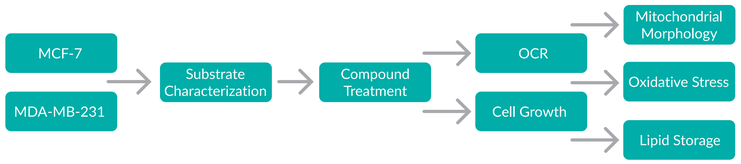
KEY FINDING

Using an assay panel to characterize metabolic changes in cell lines provides important insights into the effects of chemotherapeutics on cellular metabolism.

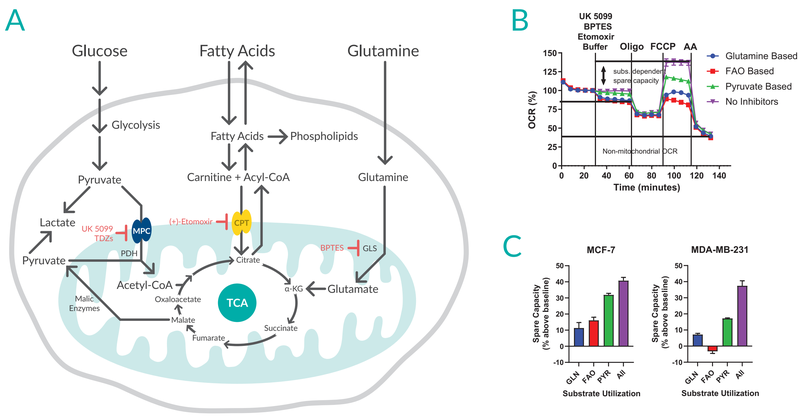
Abstract

Chemotherapeutics can have profound effects on cancer cell metabolism due to either direct or indirect effects. In many instances these effects can vary based on cell type and level of aggressiveness. Here we focus on two breast cancer cell lines MCF-7 and the triple negative MDA-MB-231 cells in a panel of assays designed to study specific aspects of cellular metabolism. These cells will be screened against three chemotherapeutics (paclitaxel, AG-879, and oxaliplatin), which have previously been shown to have effects on cellular metabolism, and the mitochondrial-targeted antioxidant mitoquinol (MitoQ), which has been shown to exhibit anticancer properties. Assays in this panel include cell growth, mitochondrial function, oxidative stress, lysosomal function, and lipid storage.

Through the utilization of this assay panel, we compare the different metabolic phenotypes of two breast cancer cell lines with varied severity. Additionally, we demonstrate the effects that a small subset of anticancer agents has across a wide range of metabolic processes.



Characterization of Substrate Preferences



A. Import of predominant mitochondrial metabolites and their respective inhibitors. B. Oxygen consumption rates of cells following treatment with etomoxir and UK 5099 (glutamine-based respiration), UK 5099 and BPTES (FAO-based respiration), etomoxir and BPTES (pyruvate-based respiration), and no inhibitor treatment (all metabolites). C. MCF-7 or MDA-MB-231 cells were incubated overnight in DMEM supplemented with 10% FBS, 1 mM pyruvate, 2 mM glutamine, and 10 mM glucose at 5% CO_2. The following day, media was exchanged for XF media with identical supplementation and a mitochondrial stress test performed as detailed in panel B above. Resulting data show spare capacity of cells respiring using glutamine, FAO, pyruvate, or all metabolites as fuel sources. All inhibitors were obtained from Cayman Chemical: BPTES (Item No. 19284), UK 5099 (Item No. 16980), (+)-Etomoxir (sodium salt) (Item No. 11969).

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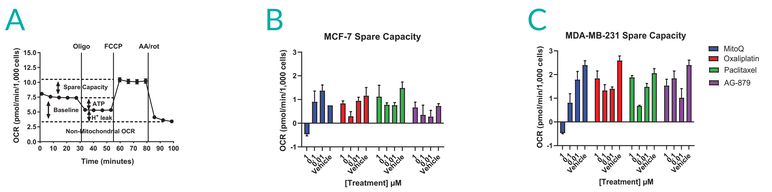
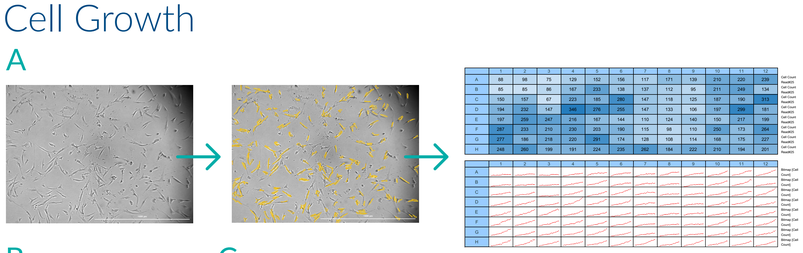
Oxygen Consumption/Mitochondrial Stress Test



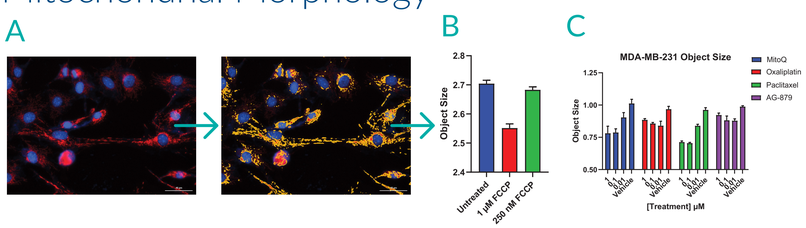
A. Mitochondrial stress test. B-C. Spare capacity of MCF-7 and MDA-MB-231 cells treated overnight with compound. Briefly, MCF-7 and MDA-MB-231 cells were treated with compound or vehicle and incubated for 18 hours in DMEM supplemented with 10 mM glucose, 1 mM pyruvate, 2 mM glutamine, and 10% FBS at 5% CO_2. The following day, media was exchanged for XF media with identical supplementation and a mitochondrial stress test was performed.

Cell Growth



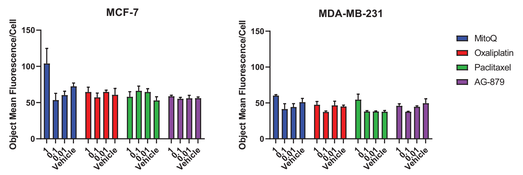
A. Probe-free cell growth monitoring. Cells were plated and allowed to settle overnight prior to treatment with compound. The following day, cells were treated with test compounds and placed in the atmospherically controlled BioSpa 8 automated cell incubator and imaged every six hours using the Cytation 5 over a five-day period. B-C. Cell number is quantified using Gen5 imaging software and growth rates are calculated. The growth rates of MCF-7 and MDA-MB-231 cells following treatment with compounds was monitored over a five-day period.

Mitochondrial Morphology



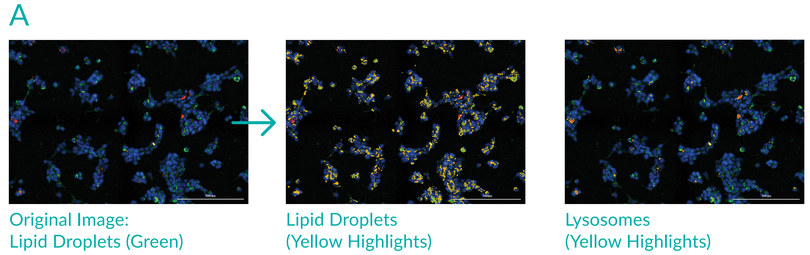
A. Cells are co-stained with Hoechst nuclear stain and either MitoLite™ Deep Red FX660 (Cayman Item No. 25160) (pictured in A) or MitoTracker Green (Thermo) and imaged using the Cytation 5 multimode imaging plate reader. B. Mitochondrial object area and size was calculated using Gen5 image analysis software. Larger objects were consistent with a more tubular mitochondrial morphology, whereas smaller object size appeared consistent with the punctate fragmented mitochondrial morphology. FCCP is used a positive control for mitochondrial fragmentation C. MCF-7 (not shown) and MDA-MB-231 cells were incubated for 24 hours in the presence of compounds and treated as in A.

Oxidative Stress



Oxidative stress was measured by staining cells with 5 µM dihydroethidium (Cayman Item No. 12013) followed by the addition of compounds. Cells were incubated in the presence of compounds for two hours, followed by staining with Hoechst nuclear stain (Cayman Item No. 600332). Cells were then imaged using the Cytation 5. Object mean fluorescence was quantified and normalized to cell number.

Metabolite Storage and Processing



A. Cells were incubated for 24 hours in the presence of compounds. Following incubation, cells were stained with the lysosomal stain LysoBrite™ Red (Cayman Item No. 25157), followed by fixation and staining with the neutral lipid stain LipidTOX™ Green (Thermo). Hoechst stain (Cayman Item No. 600332) was added as a nuclear stain. Neutral lipid staining and lysosomal staining were quantified and normalized to cell number (data not shown). B-C. Staining of neutral lipids in response to compound treatment in MCF-7 and MDA-MB-231 cells.

Conclusions

- MCF-7 and MDA-MB-231 cells have different substrate preferences and respiratory capacities.
- At high concentrations, MitoQ impairs mitochondrial function, inhibits cell growth, and increases oxidative stress.
- As anticipated, most other compounds affecting mitochondrial function also affect cell growth and mitochondrial morphology. The exception was MitoQ, which appeared to facilitate growth in MDA-MB-231 cells at 100 nM, despite limiting mitochondrial spare capacity and the observed fragmentation of mitochondria.