BACKGROUND: Cancer cells utilize aerobic glycolysis rather than oxidative phosphorylation to generate most cellular ATP (Warburg phenomenon). In non-transformed differentiated cells, the adenine nucleotide translocator (ANT) catalyzes exchange of ATP for ADP across the mitochondrial inner membrane. Bongkrekic acid (BA) and carboxyatractyloside (CATR) specifically inhibit ANT. Here, our AIM was to assess whether mitochondrial ATP translocation in cancer cells depends on ANT.

METHODS: Mitochondrial membrane potential (ΔΨ) was assessed by confocal microscopy of tetramethylrhodamine methylster (TMRM) fluorescence. Respiration by HepG2 and A549 cells was determined with a Seahorse XF-24 Analyzer. RESULTS: In rat hepatocytes, respiratory inhibition by myxothiazol (MYX) slightly decreased ΔΨ, but subsequent oligomycin (OL), BA or CATR collapsed ΔΨ, indicating that mitochondrial hydrolysis of glycolytic (cytosolic) ATP sustains ΔΨ. In HepG2 and A549 cells, MYX also slightly decreased ΔΨ, and subsequent OL collapsed ΔΨ. By contrast to hepatocytes, BA and CATR added after MYX did not collapse ΔΨ, whereas 2-deoxyglucose (2-DG), a glycolytic inhibitor, added after MYX, MYX+BA and MYX+CATR did collapse ΔΨ. OL but not BA or CATR alone decreased respiration in both cell lines, whereas BA and CATR inhibited hepatocyte respiration. ANT2 is the predominant ANT isoform expressed in cancer cells, and 2-DG after MYX in ANT2 knockdown cells depolarized mitochondria.

CONCLUSION: In cancer cells ANT is not the principal ATP transporter responsible for mitochondrial uptake of glycolytic ATP from the cytosol. Moreover, ANT2 deficiency does not alter uptake of glycolytic ATP into mitochondria. Warburg metabolism, therefore, appears to utilize an alternative pathway for entry of ATP into mitochondria.

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Metabolic Profiling of Multicell Tumor Spheroids by NADH Fluorescence and Spatially-Resolved Oximetry


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While fluorescence intensity-based metabolic imaging techniques have provided a useful means of monitoring cellular energetics, recent work has demonstrated that fluorescence lifetime imaging (FLIM) provides additional details into the subcellular trafficking of energy intermediates within the cell. Specifically, FLIM can measure the reorganization of NADH within distinct subcellular pools with change in the metabolic state induced by inhibitors, uncouplers and subcellular availability. Here, we compare NADH-intensity and FLIM measurements of metabolism of cells grown as either monolayer culture or 300-500 μm diameter multicell tumor spheroids in media under different growth conditions. These measures are correlated with cellular respiration monitored using an oxygen-sensitive electrodes to test the hypothesis that NADH FLIM-based metabolic imaging more accurately measures the cellular metabolic state of three-dimensional living tissue than intensity-based measurements.

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