Estimating and Partitioning the Flux of ATP in Cells in Culture using mol cell^{-1} s^{-1} and the Stoichiometries of Metabolism

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We have demonstrated the advantages of using moles of a substance per cell as a metric for dose/exposure in cell culture. This dosing metric greatly increases the information content of data and enhances the repeatability and quality of experimental results compared to traditional dosing metrics, such as initial extracellular concentration. However, the use mol cell^{-1} or mol cell^{-1} s^{-1} for cell culture work can be applied beyond the expression of dose of a xenobiotic. We have applied the use of mol cell^{-1} s^{-1} to generate new knowledge on the flux of ATP in cells. Using data generated on the rates of oxygen consumption (OCR), proton efflux rate (PER) and proton production rate (PPR) by cells, as quantitated by extracellular flux analyzers (*e.g.* Agilent/Seahorse Bioscience instrumentation), the flux of ATP can be estimated. The use of moles as a base unit meshes directly with the chemistry and biochemistry of the system; allows stoichiometric relationships to be revealed; and provides a universal approach for direct comparison of experimental results from different cells. This work is an advance our 2015 presentation as several unknown unknowns were identified. Examples of each of these will be presented demonstrating how using the units of mol cell^{-1} s^{-1} leads to: data that have greater information content; absolute results that can be compared directly between laboratories around the world; and advancements pushing the frontiers of Quantitative Redox Biology.

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Loss of the Nuclear Receptor Rev-erbα Results in Upregulation of Glycolysis in Mouse Embryonic Fibroblasts Exposed to Hyperoxia

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Premature neonates with immature lungs often require supplemental oxygen for survival. Hyperoxic exposure in neonates results in impaired formation of alveoli and lung vasculature that is characteristic of bronchopulmonary dysplasia (BPD), which can have long term consequences for the development of the respiratory and nervous systems. We have shown that hyperoxia reduces levels of Rev-erb*α*, a transcriptional repressor and circadian regulator of metabolism, in the mouse lung. We hypothesized that reduction of Rev-erbα expression would alter metabolism in a mouse embryonic fibroblast (MEF) model of hyperoxic exposure. In order to determine changes in metabolic function and utilization of key substrates resulting from loss of Rev-erb*α*, we exposed WT and Rev-erb*α* KO MEFs to either air or hyperoxia (95% O_2/5% CO_2) for 24 hours. Control cells were exposed to air for 24 hours. Mitochondrial respiration, glycolysis, and substrate utilization were then determined using a Seahorse XF24 Bioanalyzer. Additionally, a 4 day growth curve was performed in air to determine differences in cell proliferation. Rev-erbα KO MEFs exposed to hyperoxia exhibited increased oxidative respiration and glycolysis compared to WT cells. Substrate utilization experiments showed that immediately following hyperoxic exposure, Rev-erb*α* KO MEFs were less dependent on glutamine and fatty acids, but had a higher capacity to metabolize glucose. Rev-erb*α* KO MEFs exhibit a higher basal rate of growth in air compared to WT. In conclusion, disruption of Rev-erbα in MEFs resulted in increased glycolysis and higher levels of proliferation. We speculate that these changes may render the cells more vulnerable to oxidative stress and less viable. We will test this using protein carbonyls as a measurement of oxidative stress levels and clonogenic assays as a measurement of cell survival.

The Role of MPZL3 as a Metabolic Regulator in Ovarian Cancer

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Ovarian cancer is comprised of multiple histological subtypes that vary widely in metabolic phenotypes. Moreover, metabolic flexibility is required to sustain survival in different tumor niches during metastatic progression in the peritoneal cavity. For example, colonization of omental adipose tissues requires alterations in lipid transport, synthesis and metabolism. We previously identified a novel regulator of lipid metabolism, myelin protein zero-like 3 (MPZL3), using Mpzl3 knockout mice and C57BL/6N mice treated with Mpzl3 antisense oligonucleotides (ASO). Loss of Mpzl3 resulted in reduced adiposity, decreased serum triglycerides and cholesterol, and reduced hepatic lipid synthesis. Indirect calorimetry revealed a significant decrease in respiratory exchange ratios with Mpzl3 ASO treatment ($p<0.001$), indicating an increase in fat oxidation. Further, expression of several lipogenic enzymes, including FASN, which has been associated with ovarian cancer, was significantly downregulated in white adipose tissue of Mpzl3 knockdown animals, while tissue-specific decreases in expression of mitochondrial ferredoxin reductase (FDXR) were observed. We therefore hypothesized that Mpzl3 may be a novel regulator of tumor lipid metabolism and mitochondrial function. Semi-quantitative RT-PCR analysis revealed that Mpzl3 mRNA expression levels are highly increased (7- to 15-fold) in ovarian cancer cell lines of high-grade serous origin (OVCAR3, OVCA433, OVCA429), compared to the normal ovarian epithelium cell line NOSE007. Moreover, siRNA-mediated Mpzl3 knockdown resulted in significantly decreased clonogenicity of OVCA433 cells. Conversely, cells of clear cell carcinoma origin (ES-2, TOV-21-G) lacked detectable expression of Mpzl3, with RNA sequencing revealing a complete loss of expression of genes within the MPZL3 gene 11q23 chromosomal region. Interestingly, we previously demonstrated that OCCC and HGSA cells differ in their mitochondrial function and bioenergetic phenotype. Fuel substrate utilization (Extracellular Flux analysis) and MPZL3 cellular localization studies are currently underway to further elucidate the divergent role of MPZL3 on lipid metabolism and mitochondrial function in ovarian cancer histological subtypes.