Project title: Coordination of glutaminolysis and glycolysis in PC3 prostate cancer cells

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Abstract:

Cancer cell proliferation strongly depends on the availability of energy as well as metabolic precursors for the synthesis of cell building blocks, which implies a close coordination between the associated key pathways: cytosolic glycolysis and mitochondrial glutaminolysis. In glycolysis, tumor cells express a certain pyruvate kinase isoenzyme termed pyruvate kinase M2 (M2-PK, PKM2). In tumor cells the tetramer : dimer ratio of PKM2 regulates whether glucose is converted to pyruvate and lactate with regeneration of energy (highly active tetrameric form) or channeled into synthetic processes (nearly inactive dimeric form). In different cell culture studies an increase in intracellular ROS concentrations induced by insulin treatment of the cells, addition of H_2O_2 into the cultivation medium or hypoxia led to cysteine oxidation of the PKM2 protein, subunit dissociation of PKM2 and a decrease of PK activity which resulted in a decrease in glucose consumption and lactate production. Several studies suggest that hypoxic conditions cause increased ROS production by mitochondrial complex III. Short term incubation experiments show that during hypoxia myxothiazol, which inhibits site III_{Q0}, was shown to decrease ROS production and to block HIF-1α, whereas antimycin A, which induces superoxide production from side III_{Q0}, was characterized to increase ROS production and HIF1α In addition, S3QEL decreases complex III_{Q0} electron leak without affecting oxidative phosphorylation. Besides mitochondrial complex III also mitochondrial glycerol 3-P dehydrogenase (mG3PDH) has been shown to participate in mitochondrial ROS production to both the mitochondrial matrix as well as the intermembrane space and cytosol.

The aim of the project is to investigate the effect of long term mitochondrial complex III inhibition by antimycin A and myxothiazol as well as of mG3PDH inhibition on PKM2, the composition of the glycolytic enzyme complex, the isoenzyme equipment of lactate dehydrogenase and of the malate aspartate shuttle enzymes malate dehydrogenase and glutamate oxaloacetate transaminase as well as the coordination of glycolytic, glutaminolytic and serine conversion rates in PC3 prostate cancer cells at both 21% oxygen as well as 1.5% oxygen cultivation conditions.

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Aim: Investigate the impact of the mitochondrial glycerol-3P dehydrogenase inhibitor RH02211 and of the mitochondrial CIII inhibitor S3QEL-2 on oxygen consumption and H_2O_2 production as well as the impact of CIII inhibitors antimycin A and myxothiazol on H_2O_2 production after long term incubation (96 hours).