P797 METABOLIC PROFILING IN ERCC6L2 AND SHWACHMAN DIAMOND SYNDROME

Topic: 11. Bone marrow failure syndromes incl. PNH - Biology & Translational Research

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Background: Germline mutations in genes involved in DNA repair, telomere maintenance, and ribosome biogenesis are established causes of bone marrow failure (BMF) syndromes. Biallelic ERCC6L2 germline mutations cause BMF and accumulation of somatic TP53 mutations in bone marrow, contributing to the disease progressing into acute myeloid leukemia. ERCC6L2 is tentatively indicated in DNA repair and mitochondrial function, however, understanding its function is still in its infancy.

Aims: To particularly understand the role of mitochondrial function in ERCC6L2 syndrome, we compared cellular metabolism in ERCC6L2 syndrome and Shwachman Diamond syndrome (SDS), a ribosomopathy also prone to somatic TP53 mutagenesis. Our aim was to discover any underlying alterations in mitochondrial function and to perceive any differences between the two syndromes that are similar in phenotype but different in genotype.

Methods: To study the effect of the ERCC6L2 and SD85 germline mutations on cellular metabolism in isolation from the effect of accumulating somatic mutations in the bone marrow, we examined patient-derived skin fibroblasts (ERCC6L2 syndrome, n=2; SDS, n=2) and healthy controls (n=2). Mitochondrial function was studied with Seahorse XFe96 Analyzer where mitochondrial oxygen consumption rate (OCR) was measured under different glucose and glutamine concentrations. Furthermore, we inhibited mitochondrial pyruvate carrier (MPC) to examine the metabolic compensation for mitochondrial pyruvate deficiency via Seahorse Substrate Oxidation Stress Test. Mitochondrial DNA (mtDNA) amount was quantified in patient samples using qPCR. To further investigate the metabolism in each BMF syndrome, untargeted metabolomic screening of ERCC6L2 (n=4), SDS (n=3) and healthy control (n=3) fibroblasts in normal and low-glucose conditions was conducted by liquid chromatography mass spectrometer. We cross-referenced differentially expressed (DE) metabolites with DE genes (3’ RNAseq) in a matched set of samples to identify important biological pathways.

Results: Our results indicate impaired mitochondrial respiration under low energy availability in both ERCC6L2 syndrome and SDS, as both BMF syndromes showed decreased maximal respiratory and reserve capacity in low-glucose conditions. Equal mtDNA amount was confirmed in patient and control cells suggesting that the decline was not related to changes in mitochondrial content. In ERCC6L2, but not in SDS cells, maximal respiratory and reserve capacity improved upon the addition of glutamine. In addition, we observed a lower glutamine concentration in both ERCC6L2 and SDS in low-glucose conditions. MPC inhibition lowered the respiration in SDS cells, but not in ERCC6L2 cells, indicating that pyruvate may be compensated by other substrates, such as glutamine oxidation, in ERCC6L2 but not in SDS cells. Together, these findings imply increased consumption of glutamine in ERCC6L2 cells and possibly impaired glutamine utilization in SDS cells. Metabolomic screening and integration of metabolomics data with transcriptomics detected subtle trends indicating changes in TCA metabolite and gene expression as well as in nicotinate/nicotinamide metabolism.
Summary/Conclusion: We show altered mitochondrial function in low-glucose conditions in two BMF syndromes, and propose glutamine-dependency as a factor for challenged mitochondrial function under low energy substrate availability in ERCC6L2 syndrome. Further studies on the metabolic behavior of the hematopoietic stem cell and its niche in BMF syndromes are needed to understand the role of metabolism in the disease progression.