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Proteome and bioinformatic analyses of cellular models of methylmalonic acidemia reveal lysosomal dysregulations

Background. Methylmalonic acidemia (MMA) is the most common form of organic acidemias, an heterogeneous group of rare inherited metabolic disorders that affect amino acid and protein metabolism. As suggested by their name, organic acidemias are characterized by the accumulation of organic acids at toxic concentrations. Isolated methylmalonic acidemia is caused by impaired activity/expression of the enzyme methylmalonyl-CoA mutase (MUT). MUT deficiency determines the increase of methylmalonyl-CoA and methylmalonic acid, causing severe dysfunctions in various metabolic pathways and mitochondrial damage, with significant impacts on organs such as brain, liver, and kidneys. Owing to the complexity of the disorder, many of the systemic dysfunctions in MMA have not been fully mechanistically validated [1].

Methods. The proteomic landscape of MMA was investigated using diverse cell models. First, a MUT-knockout (MUT-KO) cell line was generated via CRISPR/Cas9 in HEK-293 cells. Also, the expression of a MUT-FLAG protein was rescued in these cells (MUT-Rescue). Finally, dermal fibroblasts derived from MMA patients were collected and cultured. Through the combination of high-resolution proteomics and bioinformatics analysis, the whole cell proteomes and their sub-proteomes were characterized [2] to refine and uncover new pathologic mechanisms connected with MUT deficiency.

Results. In this study, a data-independent acquisition (DIA) proteomic approach was used to simultaneously compare the proteomes of the three cell lines: WT, MUT-KO and MUT-Rescue. Profile analysis was performed to select the significant proteins according to their trend of regulation: indeed, two clusters of proteins were highlighted as the most significantly changing (up or down) in MUT-KO compared to WT and MUT-Rescue. Gene Set Enrichment analysis (GSEA) of the whole proteome dataset enriched several terms positively correlated with the MUT-KO condition and which correspond with several known features of MMA pathophysiology, including oxidative phosphorylation and lysosomal dysfunction. However, two of the most interesting dysregulations not yet associated with MUT deficiency occurred for lysosome-associated membrane glycoprotein 2 (LAMP2) and stathmin (STMN1), which both showed decreased signals in MUT-KO cells. To corroborate these findings, we explored the proteome of MMA fibroblasts and found down-regulated proteins common to the MUT-KO model, such as LAMP2. Being LAMP2 a component of the lysosomal membrane, we investigated possible changes in the levels of other lysosomal and autophagy markers in MMA fibroblasts, revealing up-regulation of LAMP1, LC3 and its cleaved form LC3-II, and p62. To elucidate the effects of MUT deficiency on lysosomal and autophagy regulation, we also analyzed the morphology and functionality of MMA-lysosomes that showed deep alterations. At the same time, also the measurement of the autophagic flux in MMA cells resulted strongly impaired. Notwithstanding, the treatment with an anti-propionigenic drug was capable of totally rescuing lysosomal morphology and functional activity in MUT-deficient cells. These results indicate a strict connection between MUT deficiency and lysosomal-autophagy dysfunction.

Conclusions. Our proteomic data [3] show that MUT deficiency is connected with profound proteome dysregulations, revealing molecular actors involved in lysosome and autophagy functioning. In this study, we demonstrate that MUT-deficient cells have defective homeostatic mechanisms in the regulation of autophagy and lysosome functions, demonstrating that MMA triggers such dysfunctions impacting on autophagosome-lysosome fusion and lysosomal activity.

References.

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