DOI 10.18699/SBB-2022-642

The emerging role of SIRT6 in the mitochondrial regulation

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Key words: SIRT6, mitochondria, cellular metabolism

Motivation and Aim: SIRT6, a member of the SIRTUIN family, is localized in the nucleus and regulates many intracellular processes through two main enzymatic functions – histone deacylation and mono-ADP-ribosylation. SIRT6 is implicated in genomic stability, DNA repair, telomere maintenance, cellular metabolism and, importantly, has critical roles in the brain protecting against aging-associated diseases [1]. Moreover, SIRT6 deficiency increases mitochondrial defects, including mtDNA damage accumulation and impaired mitochondrial dynamics in the heart and skeletal muscles [2, 3]. However, despite many promising studies in recent years, the molecular mechanisms underlying SIRT6 impact on mitochondrial function in the brain are still poorly understood. In this work, we examined the SIRT6-induced changes in mitochondrial activity by comparing transcriptomic and metabolomic profiles of brain-specific SIRT6 knockout mice (SIRT6-KO) and wild-type mice (WT).

Methods and Algorithms: Generation of brain-specific SIRT6 conditional knockout mice, library preparation and RNA sequencing steps were performed in the Dr. Toiber laboratory [4] at the Ben-Gurion University of the Negev in Israel. Raw RNA reads of 22 mouse samples were filtered and trimmed using *fastp* [5] and then processed via version 3.0 of the *nf-core/rnaseq* pipeline [6]. Normalization and differential gene expression (DGE) analysis were performed via the *DESeq2* [7] package in the R programming environment. WT and SIRT6-KO samples from the mouse brain tissue were also collected for the liquid chromatography coupled with mass spectrometry (LC-MS) metabolomics experiment. Obtained metabolomics dataset was processed using an in-house script and analyzed using *MetaboAnalyst* software [8]. Differentially accumulated metabolites (DAMs) were defined according to the following criteria: |Fold Change| > 1.5 and p-value < 0.05.

Results: Differential expression analysis of WT vs. SIRT6-KO samples revealed 217 significant genes related to essential mitochondrial processes, including oxidative phosphorylation, mitochondrial import, and tricarboxylic acid cycle. Importantly, down-regulated genes constituted a substantial proportion of all differentially expressed mitochondria-related features, exceeding 91 % of genes. Additionally, we found that the deficiency of SIRT6 leads to decreased mitochondrial content and reduced mitochondrial membrane potential. In line with transcriptomic results, preliminary analysis of mass spectrometry data of SIRT6-KO mouse brains revealed 103 (out of 235) metabolites differentially accumulated in the SIRT6-KO mice compared to the control group. Among them are NAD^+ (p-value = 0.0011), malate (p-value = 0.0012), fumarate (p-value = 0.0015), NADH (p-value = 0.0100), and alpha-ketoglutarate (p-value =

= 0.0194) metabolites that were previously shown to play a direct role in the oxidative phosphorylation process [9]. Taken together, our results emphasize the crucial role of SIRT6 in the essential mitochondrial processes and provide new insights into its potential targets.

Acknowledgements: The research was funded by RFBR and Moscow City Government according to the project No. 21-34-70051.

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