CHRD 2024: Abstract Submission Form

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Presenter Status
Post-Doctoral Fellows

Role in the project
Analyze Data

Research Category
Basic Science

Title
Decreased activity of tissue non-specific alkaline phosphatase in patients with hypophosphatasia causes metabolic reprogramming and mitochondrial dysfunction leading to development of extra-skeletal manifestations

Background
Hypophosphatasia (HPP) is an inherited disorder of skeletal mineralization affecting primarily bones and teeth and is characterized by low circulating levels of tissue non-specific alkaline phosphatase (TNSALP). Patients with HPP also have extra-skeletal manifestations, including muscle weakness, muscle and joint pain, CNS deficits and seizures. Biallelic pathogenic variants in ALPL, which encodes TNSALP, cause HPP. Recent evidence suggests that decreased TNSALP is linked to mitochondrial dysfunction, which may cause extra-skeletal manifestations in HPP patients.

Objective
To understand the pathogenesis of the skeletal and non-skeletal manifestations of hypophosphatasia.

Methods
We tested this hypothesis in induced pluripotent stem cells (iPSCs) generated from three HPP patients. Briefly, patient peripheral blood mononuclear cells were reprogrammed into iPSCs using Sendai virus Cytotune 2.0 kit. The iPSCs were characterized for pluripotency and ALPL variants. These iPSCs were then differentiated into patient-specific osteoblasts and characterized. Mitochondrial function was assessed in iPSCs and osteoblasts using biochemical assays, gene expression analysis and Seahorse respiration analysis. A comprehensive metabolomics analysis was used to understand the cellular metabolism arising from faulty TNSALP and the impact on osteoblast development.

Results
We report the generation of three independent HPP patient-specific iPSC lines with compound heterozygous pathogenic variants in ALPL. These lines were positive for pluripotency markers. We observed significantly delayed osteoblast differentiation in HPP patient lines confirmed by Alizarin red and alkaline phosphatase staining and gene expression analysis for osteoblast markers. Mitochondrial dysfunction in the patient-specific osteoblasts was confirmed by significantly reduced mitochondrial respiration using Seahorse analysis. Alterations in mitochondrial gene expression was also observed using real-time PCR. Metabolomics identified downregulation of the citric acid cycle, pyruvate metabolism and fatty acid oxidation, which indicate intrinsic mitochondrial dysfunction.

Conclusion
We have successfully established HPP patient-specific iPSC models. Cellular assessment of these iPSC lines confirmed the presence of energy deficits, metabolic reprogramming, and mitochondrial dysfunction during bone development, likely contributing to the extra-skeletal manifestations of HPP.

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