NOTCH1 mitochondria localization during heart development promotes mitochondrial metabolism and the endothelial-to-mesenchymal transition in mice.

Wang, J., Zhao, R., Xu, S. et al. Nat Commun 15, 9945 (2024).

Presenter: Chun-Han Chu **Date/Time:** 2025/03/20, 15:20-16:10pm
Commentator: Dr. Yen-Wen Liu **Location:** Rm. 601, Med College Building

Background:

Endothelial-to-mesenchymal transition (EndMT) was initially observed during endocardial cushion formation in embryonic valvuloseptal morphogenesis. Impaired EndMT can lead to various congenital heart defects (CHDs) including tetralogy of Fallot (TOF), atrioventricular septal defect, and bicuspid aortic valve (BAV). Several signaling pathways, including Notch, bone morphogenetic proteins (BMP), and transforming growth factor β (TGF-β), regulate EndMT. Recent studies indicate that endothelial cells (ECs) rely more on glycolysis than mesenchymal cells. Moreover, impaired fatty acid metabolism in endothelial cells within the mitochondria can induce EndMT. However, the mechanism by which metabolism regulates EndMT remains unclear.

NOTCH1 as a receptor in Notch signaling, regulating EndMT and serving as the most commonly mutated gene in TOF. NOTCH1 intracellular domain (NICD1), the activated form of NOTCH1, is produced via a two-step proteolytic cleavage, following membrane receptor-ligand interaction. Recent research has also revealed the mitochondrial localization of NICD1 in M1 macrophages and triple-negative breast cancer cells, suggesting a potential metabolic function. Furthermore, mouse models with NOTCH1 mutations exhibit an exacerbated CHD phenotype when exposed to maternal diabetes, which also implies that there may be a link between NOTCH1, metabolism, and EndMT?

Objective:

To investigate the role of NICD1 in the mitochondria of developing mouse fetal hearts and its induction of EndMT through the mitochondrial metabolism.

Results:

The authors first used mitochondrial fractionation and found that NICD1 is prominently localized in the mitochondria of cardiac ECs at E11.5. Mutagenesis revealed that NICD1 translocates to the mitochondrial matrix via an internal signal. Mass spectrometry and bimolecular fluorescence complementation (BiFC) assays identified PDHB as a direct binding partner of NICD1, with the N-terminal to the second nuclear localization signal region being essential for this interaction.

A mitochondria-targeted NICD1 (mitoNICD1) was then constructed and shown to activate PDH by binding PDHB independently of transcription. Metabolic flux and Seahorse Mito Stress tests demonstrated that mitoNICD1 enhances TCA cycle activity, oxygen consumption, and ATP production. Mechanistically, NICD1 inhibits access of PDK1–4 and PDP1 to PDHA1, reducing its phosphorylation and activating PDH. This activation induces EndMT, which can be blocked by PS-48 and mimicked by Dichloroacetate (DCA).

Clinically, Sanger sequencing of 300 TOF patients revealed 15 NOTCH1 missense mutations, five within the NICD1 region. The R2263Q mutation, adjacent to the mitochondrial entry signal, disrupted NICD1 mitochondrial localization and PDHB interaction, leading to impaired PDH activation and increased CHD risk.

Conclusion:

This study found that NICD1 is enriched in the mitochondria of developing mouse fetal hearts and PDH activation contributes to NICD1-induced EndMT. NOTCH1 mutations in TOF patients impair mitochondrial translocation, thus lowering PDH activity.