111. Phosphorylation of Novel Tyrosine Residues in CKMT2 Confers Protection Against Hypoxia/Reoxygenation Injury

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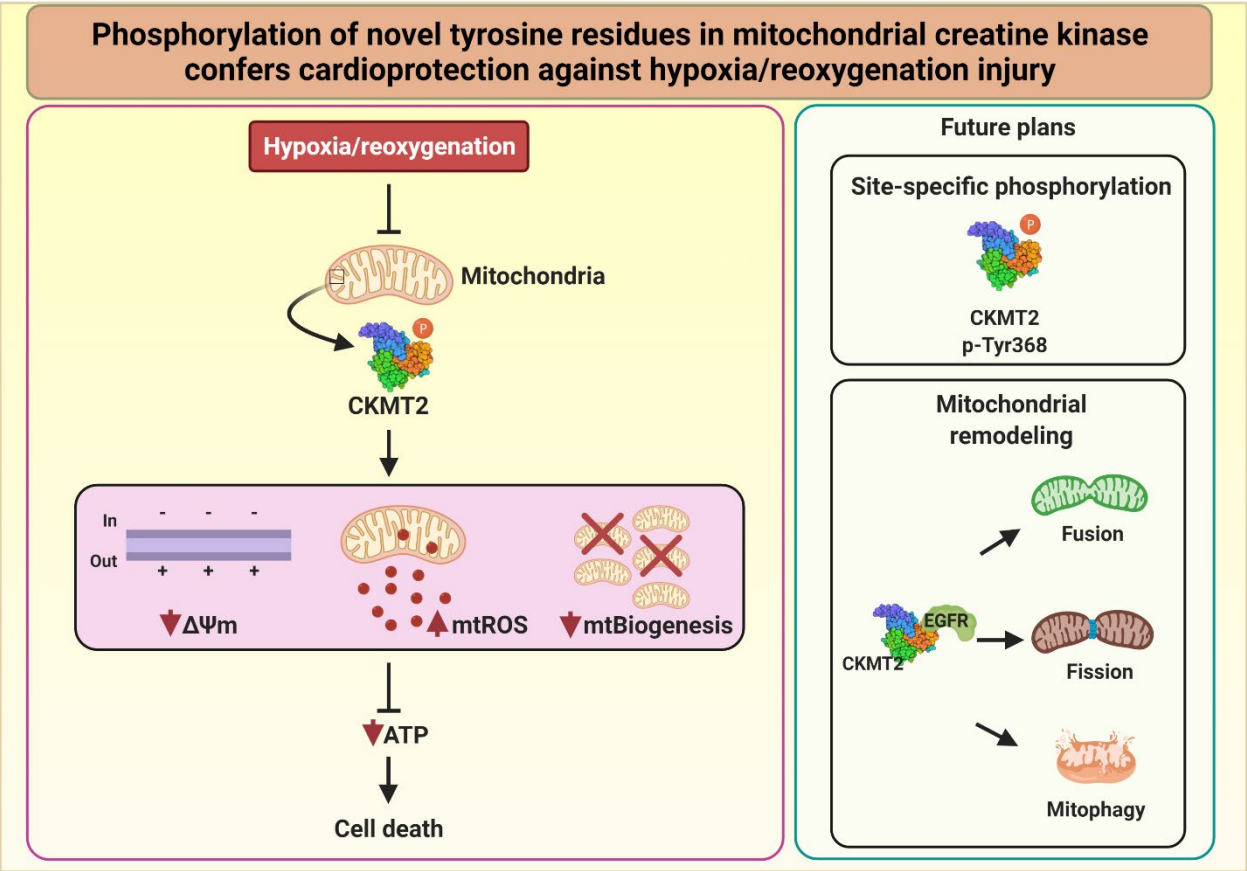
Background: Proteomic studies have demonstrated ischemic preconditioning (IPC) can assert cardioprotection during ischemic cardiomyopathy (ICM). Considering IPC occurs briefly, protein expression is also rapidly regulated, indicating the importance of protein modulation by post-translational modifications. This study aimed to identify and analyze novel phosphorylated mitochondrial proteins that can be targeted to address ischemia/reperfusion (I/R) injury.

Methods: Sprague-Dawley rat hearts were used in an ex vivo Langendorff system to simulate normal perfusion, I/R, and IPC condition, after which the samples were prepared for phosphoproteomic analysis. Employing human cardiomyocyte AC16 cells, we investigated the cardioprotective role of CKMT2 through overexpression and how site-directed mutagenesis of putative CKMT2 phosphorylation sites (Y159A, Y255A, and Y368A) can affect cardioprotection by measuring CKMT2 protein activity, mitochondrial function and protein expression changes.

Results: Phosphoproteomics revealed dephosphorylation of mitochondrial creatine kinase (CKMT2) during ischemia and I/R in a rat model while preserving its phosphorylated state during IPC. Using human ventricular cell line AC16, CKMT2 overexpression conferred cardioprotection against hypoxia/reoxygenation (H/R) by increasing cell viability and mitochondrial ATP, preserving mitochondrial membrane potential, and reducing ROS generation, while phosphomutations, especially in Y368, nullified cardioprotection by reducing cell viability and increasing ROS production during H/R. CKMT2 overexpression increased mitochondrial function by mediating the PGC1α/ERRα axis, and these effects were mostly inhibited by Y368A mutation.

Conclusion: Regulation of quantitative expression and phosphorylation site Y368 of CKMT2 offers a unique mechanism in future ICM therapeutics.



Clinical Implications: My study will help enable cardiovascular clinicians to determine a novel target that offers a different perspective on cardioprotection during I/R and H/R injury rather than the conventional method of targeting infarct size-reduction, presenting fresh insights in developing future therapeutic strategies for ICM.