Impaired mitochondrial oxidative phosphorylation and fatty acid oxidation with enhanced mitochondrial oxidative stress in spontaneously-occurring feline hypertrophic cardiomyopathy
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Published in:
Journal of Cardiac Failure

DOI:
10.1016/j.cardfail.2014.07.098

Publication date:
2014

Document Version
Early version, also known as pre-print

Citation for published version (APA):
https://doi.org/10.1016/j.cardfail.2014.07.098
**O-005**

A Noble Target Molecule of Nobiletin is Crucial for Cardiac Hypertrophic Responses

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Introduction: Maladaptive hypertrophy is being recognized as a critical event during the development of heart failure. We found that nobiletin, a poly-methoxy flavonoid derived from Citrus unshu, repressed hypertrophic responses in cardiomyocytes and prevented the development of heart failure after myocardial infarction in rats. Thus, nobiletin is expected as a useful agent for heart failure. However, the target molecule of nobiletin in cardiomyocytes is still unclear. Methods and Results: First, we investigated the localization of nobiletin within cardiomyocytes by generating nobiletin conjugated with Tokyo-green, a fluorescein analogue (TG-nobiletin). We confirmed that TG-nobiletin retains its ability to inhibit cardiomyocyte hypertrophy in a manner similar with natural nobiletin, and found TG-nobiletin was distributed in a cytoplasm of cardiomyocytes. Next, to identify nobiletin-binding proteins (NBPs), we synthesized biotin-conjugated nobiletin (Bio-nobiletin). Protein extracts from rat hearts were incubated with Bio-nobiletin or Biotin alone and binding proteins were precipitated with streptavidin beads. By mass-spectrometric analysis, we identified 162 novel NBPs in the heart. In addition, Bio-nobiletin pull-down assay showed that Bio-nobiletin could interact with recombinant NBPI, which related to metabolic processes. In cultured cardiomyocytes, knockdown of NBPI lost the inhibitory effects of nobiletin against phenylephrine-induced cardiomyocyte hypertrophy. Conversely, overexpression of NBPI inhibited phenylephrine-induced hypertrophy. Conclusions: These results suggest that NBPI may be a novel molecular target of nobiletin, a potent therapeutic agent for cardiac hypertrophy.

**O-007**

Mitochondrial Function as a Potential Mediator of Substrate Switching in the Heart

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Purpose: We previously reported that glucose uptake increased markedly and fatty acid uptake decreased at the congestive heart failure (CHF) stage, associated with decreased gene expression related to mitochondrial function in a rat model of heart failure. The purpose of the study was to clarify the link between the changes of substrate utilization and mitochondrial function. Methods and Results: Cardiomyocyte function was determined by mitochondrial membrane potentials, decreased ATP caused by CCCP injection means dispersion of the mitochondrial function. In ex vivo perfused hearts, CCCP also decreased the mitochondrial membrane potential assessed by two-photon laser microscopy. The glucose uptake increased 1.96 fold compared to the vehicle group and the fatty acid uptake decreased 0.48 fold 90 minutes after CCCP administration, indicating that mitochondrial dysfunction caused the changes in substrate uptake. Conclusions: This rat model using CCCP could be used as a model of mitochondrial dysfunction. Mitochondrial dysfunction may be closely associated with metabolic substrate switching in the heart, especially in the failing heart.

**O-008**

Impaired Mitochondrial Oxidative Phosphorylation and Fatty Acid Oxidation with Enhanced Mitochondrial Oxidative Stress in Spontaneously-occurring Feline Hypertrophic Cardiomyopathy

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Mitochondria play a crucial role in the development of various cardiovascular diseases. The aim of the study was to elucidate the role of mitochondrial dysfunction and oxidative stress in hypertrophic cardiomyopathy (HCM). The cardiac and skeletal muscle were obtained from 9 spontaneously-occurring dilated cardiomyopathy (DCM) cats, which were analyzed by high-resolution respirometry, and reactive oxygen species (ROS) generation originated from isolated mitochondria was assessed by using spectrophotometer. The heart extracts form rat hearts were incubated with Bio-nobiletin or Biotin alone and binding proteins were precipitated with streptavidin beads. By mass-spectrometric analysis, we identified 162 novel NBPs in the heart. In addition, Bio-nobiletin pull-down assay showed that Bio-nobiletin could interact with recombinant NBPI, which related to metabolic processes. In cultured cardiomyocytes, knockdown of NBPI lost the inhibitory effects of nobiletin against phenylephrine-induced cardiomyocyte hypertrophy. Conversely, overexpression of NBPI inhibited phenylephrine-induced hypertrophy. Conclusions: These results suggest that NBPI may be a novel molecular target of nobiletin, a potent therapeutic agent for cardiac hypertrophy.

The cardiac and skeletal muscle were obtained from 9 spontaneously-occurring dilated cardiomyopathy (DCM) cats with preserved systolic function and 13 age-matched control cats. The capacities of mitochondrial oxidative phosphorylation (OXPHOS) and fatty acid oxidation were assessed with preserved muscle fibers and isolated mitochondria by using high-resolution respirometry. Reactive oxygen species (ROS) generation originated from isolated mitochondria was assessed by using spectrophotometer. The heart extracts form rat hearts were incubated with Bio-nobiletin or Biotin alone and binding proteins were precipitated with streptavidin beads. By mass-spectrometric analysis, we identified 162 novel NBPs in the heart. In addition, Bio-nobiletin pull-down assay showed that Bio-nobiletin could interact with recombinant NBPI, which related to metabolic processes. In cultured cardiomyocytes, knockdown of NBPI lost the inhibitory effects of nobiletin against phenylephrine-induced cardiomyocyte hypertrophy. Conversely, overexpression of NBPI inhibited phenylephrine-induced hypertrophy. Conclusions: These results suggest that NBPI may be a novel molecular target of nobiletin, a potent therapeutic agent for cardiac hypertrophy.

**O-009**

Mst1 Plays a Cell-protective Role in the Heart Through FoxO1 and C/EBP-β Phosphorylation

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Mst1 kinase promotes apoptosis, thereby playing a crucial role in mediating cardiac dysfunction in response to stress. Mst1 also stimulates a cell-protective mechanism through FoxO1 as negative feedback to counteract its pro-apoptotic actions. In order to clarify how Mst1 mediates this cell-protective mechanism in the heart, Tg-DN-Mst1 were subjected to 2-h of ischemia. Tg-DN-Mst1 hearts exhibited a significantly greater infarcted area than wild-type hearts, suggesting that the cardio-protective effect of Mst1 dominates the pro-apoptotic effect. FoxO1 upregulated antioxidant genes, including catalase, and inhibited pro-apoptotic genes, including FasL, in cardiomyocytes, and these effects upon gene expression were attenuated when endogenous Mst1 was inhibited. Chromatin immunoprecipitation assays revealed that Mst1 inhibits binding of FoxO1 to the FasL promoter, but promotes binding of FoxO1 to the catalase promoter. Reporter gene assays indicated that the C/EBP-β binding element in the catalase promoter, but not the FoxO1 binding element, is critical for Mst1-mediated catalase gene upregulation. Interaction between FoxO1 and C/EBP-β was enhanced in the presence of Mst1. Mass spectrometry showed that Mst1 phosphorylates C/EBP-β at Thr299. Phosphorylation of C/EBP-β by Mst1 promoted binding of C/EBP-β to DNA. Knockdown of C/EBP-β reversed the protective effects of FoxO1 against Mst1-induced apoptosis. In summary, Mst1-mediated phosphorylation of both FoxO1 and C/EBP-β stimulates a cell-protective mechanism by facilitating FoxO1-C/EBP-β interaction and C/EBP-β-mediated transcription in the heart.

**O-010**

Wnt-β-catenin Signaling Promotes Heart Failure-Induced Skeletal Myopathy through Direct Interaction with FoxO

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Heart failure-induced skeletal myopathy is a major cause of exercise intolerance in the patients of chronic heart failure (CHF). During skeletal myopathy, muscle fiber type shifts from type I fatigue resistant fiber toward type IIb fatigable fiber. Forkhead box O (FoxO) transcription factors have been reported to mediate skeletal myopathy in CHF. We recently reported that serum from CHF model mice activated Wnt/β-catenin signaling more potently than serum from normal mice. Recent studies have revealed that β-catenin activates FoxO signaling as well as TCF-mediated canonical Wnt signaling. Here, we investigated the molecular mechanism of skeletal myopathy during CHF. We used dilated cardiomyopathy (DCM) model mice caused by troponin C2C12 myotubes. These results suggest that increased serum Wnt activity in CHF promotes skeletal myopathy through direct interaction with FoxO signaling. Our findings may provide new therapeutic targets of skeletal myopathy in CHF.