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学 位 論 文 題 目　Role of Reactive Sulfur Species in Mitochondrial Quality Control and Ischemic Stress Resistance in Rodent Hearts

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博士論文の要旨

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論文題目　Role of Reactive Sulfur Species in Mitochondrial Quality Control and Ischemic Stress Resistance in Rodent Hearts

Mitochondria are the organelles that produce adenosine 5'-triphosphate, the major energy molecule for cardiomyocytes, utilizing highly redox (reduction-oxidation)-dependent oxidative phosphorylation system. Mitochondria constantly undergo fission and fusion to maintain their integrity, which is critical for cardiac energy homeostasis and physiological function. In failing hearts, mitochondria in the cardiomyocytes are often found to be fragmented, and accumulating evidence suggests that mitigating mitochondrial hyperfission during heart failure ameliorates cardiac function. However, its molecular mechanism and practical therapeutic approach remain to be fully elucidated.

In the first section, I examined the effect of cilnidipine, one of the approved anti-hypertensive drugs, on mitochondrial fragmentation induced by myocardial infarction (MI) in mice, based on our previous *in vitro* study showing that cilnidipine decreased mitochondrial fragmentation after hypoxic stress. I revealed that treatment of cilnidipine 1 week after MI improved cardiac contractile dysfunction, suggesting that the maintenance of mitochondrial quality control by cilnidipine treatment can be an attractive therapeutics for ischemic heart failure.

In the second section, I investigated the pathophysiological role of redox-dependent mitochondrial fragmentation in mouse hearts. Our laboratory newly found that dynamin-related protein 1 (Drp1) changes its activity for promoting mitochondrial fission depending on the redox modification on its cysteine residue, such as

polysulfidation and depolysulfidation. Indeed, methylmercury (MeHg), an environmental potent electrophile that is known as a cause of Minamata disease with severe neurotoxicity, induces depolysulfidation of polysulfidated Drp1, thereby activating Drp1, promoting mitochondrial hyperfission, and exacerbating pressure overload-induced heart failure. Moreover, sulfide donor restores Drp1 polysulfidation. To test whether polysulfidation state of Drp1 is a risk factor for cardiac diseases, I examined the effect of sulfide donor on the pressure overload-induced heart failure model *in vivo* where the cardiac function was worsened by MeHg exposure. I found that treatment of sulfide donor sodium hydrogen sulfide rescued MeHg-induced cardiac vulnerability to pressure overload, suggesting that modification of Drp1 polysulfidation is important for mitochondrial quality control and cardiac robustness.

Protein polysulfidation is mediated by reactive sulfur species (RSS), which have highly reactive sulfur atoms. Due to its high reactivity, it has been suggested that RSS have important roles not only in protein polysulfidation but also in highly redox-active mitochondrial respiration, which means RSS may have critical role for maintaining mitochondrial function during the myocardial ischemic stress. Therefore, I focused on the contribution of RSS to myocardial ischemic stress resistance. This study revealed that RSS level was decreased in myocardial ischemia reperfusion (I/R) injury in mice. In neonatal rat cardiomyocytes (NRCMs), hypoxic stress-induced RSS decrease was accompanied with increase in hydrogen sulfide (H_2S), suggesting that RSS were reduced to H_2S under hypoxic condition. Treatment of RSS donor sodium tetrasulfide recovered cardiac function in I/R-induced heart failure, indicating that RSS is protective against cardiac ischemic stress. mRNA level of cysteinyl aminoacyl tRNA synthetase (CARS) 2 that is one of the RSS-producing enzymes was found to be decreased after I/R injury. Moreover, CARS2 heterozygous deleted mice were more vulnerable to I/R injury, where cardiac RSS level was significantly decreased compared to wild type mice, suggesting that RSS synthesized by CARS2 contribute to cardiac ischemic stress resistance.

Moreover, CARS2 knockdown in NRCMs declined mitochondrial membrane potential after hypoxic stress, indicating that CARS2 is required for maintenance of mitochondrial quality during hypoxic stress. I also found that mRNA level of RSS-catabolizing enzymes was decreased in I/R-injured hearts. Furthermore, it was revealed that mRNA level of cytosolic isoform of CARS2, CARS1, was increased in I/R-injured hearts. These results suggest that overall RSS synthesis and catabolism in cells contribute to ischemic stress resistance.

Collectively, these results suggest that RSS synthesis and catabolism contribute to cardiac homeostasis through protein polysulfidation and the maintenance of mitochondrial quality control, thereby inducing cardioprotective effect. The present study proposes that RSS are the key factors, along with reactive oxygen species and reactive nitrogen species, for understanding the molecular mechanism of cardiac pathophysiology. Moreover, this study suggests that the contribution of RSS to mitochondrial respiration (sulfur respiration) in the heart, proposing new insights for cardiac redox biology.

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Results of the doctoral thesis defense

博士論文審査結果

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Title
論文題目　　Role of Reactive Sulfur Species in Mitochondrial Quality Control and Ischemic Stress Resistance in Rodent Hearts

近年、活性硫黄分子種（Reactive Sulfur Species: RSS）とよばれる硫黄が直鎖状に連結した分子種が、普遍的な生命素子として生体内で豊富に産生され、エネルギー代謝やシグナル伝達を支える重要な電子移動媒体となることが示されてきた。本論文で、出願者は、虚血や圧負荷といったストレスに対する心臓の機能維持にRSSおよびRSS代謝系がどのように関与するかについてマウスを用いて検討した。

まず、心筋梗塞後の心筋細胞でミトコンドリアが異常に分裂していることに着目し、ミトコンドリアの分裂を促進するGTP結合タンパク質dynamin-related protein 1 (Drp1)の活性化を阻害する作用をもつ薬剤シルニジピンの投与が、マウス心筋梗塞モデルにおいてミトコンドリアの過剰分裂を抑制し、心機能を回復させる方向にはたらくことを示した。また、環境親電子物質であるメチル水銀をマウスに曝露すると、ポリ硫黄化されているDrp1の脱硫黄化と圧負荷による心機能の低下が誘導されるが、出願者は、脱硫黄化されたDrp1をポリ硫黄化させる作用をもつ硫化水素ナトリウムの投与により、メチル水銀を曝露した心臓に圧負荷を与えたマウスにおける心不全が有意に改善されることを見出した。以上より、心臓の機能維持にDrp1タンパク質のポリ硫黄化の重要性が示唆された。次に、出願者は、低分子の活性硫黄分子種が心筋の頑健性維持に及ぼす影響を調べた。マウス心臓の虚血再灌流モデルにおけるRSS検出蛍光指示薬を用いたイメージングの結果、低酸素下の心筋細胞や虚血心筋組織でRSSが減少し、一方、還元代謝物である硫化水素が蓄積していることが明らかになった。そこで、心臓の虚血領域にRSS供与体である四硫化ナトリウムを局所投与したところ、虚血再灌流後の心機能低下と心筋壊死が顕著に改善された。さらに、出願者は、心筋細胞の主たるシステインパースルフィド生成酵素であるミトコンドリア局在型システイニルtRNA合成酵素（CARS2）に着目し、そのヘテロ欠損マウスと野生型マウスを用いて虚血再灌流モデルにおける心臓の変化を解析した。その結果、CARS2ヘテロ欠損マウスでは野生型マウスと比較して心筋のRSS量が減少し、壊死が増加し、心不全が悪化していた。虚血再灌流後の心臓ではCARS2やRSS生成・代謝に関わる酵素のmRNA量が総じて低下していた。一方、虚血再灌流後の心臓やCARS2ヘテロ欠損心臓で細胞質局在型CARS1のmRNA量が増加し、RSS代謝リモデリングが起きている可能性が示された。

以上の結果は、タンパク質ポリ硫黄鎖やCARS2に由来する活性硫黄分子種の生成と代謝が、ミトコンドリアの恒常性制御を介して心筋の頑健性維持に関与すること個体レベルで示したものである。本成果は、哺乳類の心臓における硫黄代謝の病態生理学的意義を示した重要な知見であり、審査委員全員一致で博士学位論文に相応しいと結論した。