論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	櫃本 竜郎
論文題名 Title	Restoration of Cardiac Myosin Light Chain Kinase Ameliorates Systolic Dysfunction by Reducing Superrelaxed Myosin (心臓特異的ミオシン調節キナーゼの回復は、SRX状態のミオシンを減少させることによって収縮機能不全を改善する)

論文内容の要旨

〔Purpose〕

Background: Cardiac-specific myosin light chain kinase (cMLCK), encoded by MYLK3, regulates cardiac contractility through phosphorylation of ventricular myosin regulatory light chain. However, the pathophysiological and therapeutic implications of cMLCK in human heart failure remain unclear. We aimed to investigate whether cMLCK dysregulation causes cardiac dysfunction and whether the restoration of cMLCK could be a novel myotropic therapy for systolic heart failure.

〔Methods〕

We generated the knock-in mice (Mylk3+/fs and Mylk3fs/fs) with a familial dilated cardiomyopathy-associated MYLK3 frameshift mutation (MYLK3+/fs) that had been identified previously by us (c.1951-1G>T; p.P639Vfs*15) and the human induced pluripotent stem cell-derived cardiomyocytes from the carrier of the mutation. We also developed a new small-molecule activator of cMLCK (LEUO-1154).

〔Results〕

Both mice (Mylk3+/fs and Mylk3fs/fs) showed reduced cMLCK expression due to nonsense-mediated messenger RNA decay, reduced MLC2v (ventricular myosin regulatory light chain) phosphorylation in the myocardium, and systolic dysfunction in a cMLCK dose-dependent manner. Consistent with this result, myocardium from the mutant mice showed an increased ratio of cardiac superrelaxation/disordered relaxation states that may contribute to impaired cardiac contractility. The phenotypes observed in the knock-in mice were rescued by cMLCK replenishment through the AAV9_MYLK3 vector. Human induced pluripotent stem cell-derived cardiomyocytes with MYLK3+/fs mutation reduced cMLCK expression by 50% and contractile dysfunction, accompanied by an increased superrelaxation/disordered relaxation ratio. CRISPR-mediated gene correction, or cMLCK replenishment by AAV9_MYLK3 vector, successfully recovered cMLCK expression, the superrelaxation/disordered relaxation ratio, and contractile dysfunction. LEUO-1154 increased human cMLCK activity ≈2-fold in the Vmax for ventricular myosin regulatory light chain phosphorylation without affecting the Km. LEUO-1154 treatment of human induced pluripotent stem cell-derived cardiomyocytes with MYLK3+/fs mutation restored the ventricular myosin regulatory light chain phosphorylation level and superrelaxation/disordered relaxation ratio and improved cardiac contractility without affecting calcium transients, indicating that the cMLCK activator acts as a myotrope. Finally, human myocardium from advanced heart failure with a wide variety of causes had a significantly lower MYLK3/PPP1R12B messenger RNA expression ratio than control hearts, suggesting an altered balance between myosin regulatory light chain kinase and phosphatase in the failing myocardium, irrespective of the causes.

〔Conclusion〕

cMLCK dysregulation contributes to the development of cardiac systolic dysfunction in humans. Our strategy to restore cMLCK activity could form the basis of a novel myotropic therapy for advanced systolic heart failure.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文で研究対象としているのは、大阪大学にて発見された心臓特異的ミオシン軽鎖キナーゼ（cMLCK）である。櫃本氏はcMLCKの部分欠損したヒト症例が重症の心不全をきたしたことから、その患者のIPS細胞を作成し心筋細胞に分化させcMLCK欠損による表現型解析を進めた。結果、cMLCKの欠損がミオシンの構造変化をきたして心筋収縮性を低下させることを明らかにした。更にはcMLCKの基質であるMLCのリン酸化レベルがヒト拡張型心筋症患者で低下していることを見出し、cMLCK活性化が心不全治療に使用できる可能性を見出した。そして、cMLCKの活性アッセイ系を立ち上げヒット化合物を得て、心不全治療薬として同定した。創薬標的の分子機構の解明から化合物同定まで至った研究経過を記載した本論文は循環器系でもっとも権威あるCirculation誌に掲載された。

本論文を持って十分学位に値すると考える。