京都大学	博士（ 医 学 ）	氏 名	田 雨
論文題目	Immunosuppressants Tacrolimus and Sirolimus revert the cardiac antifibrotic properties of p38-MAPK inhibition in 3D-multicellular human iPSC-heart organoids （免疫抑制剤タクロリムスとシロリムスは、iPS 細胞由来 3D 多細胞心臓オルガノイドにおける p38-MAPK 阻害による抗線維化効果を打ち消す）		

（論文内容の要旨）

Cardiac reactive fibrosis is a fibroblast-derived maladaptive process to tissue injury that exacerbates an uncontrolled deposition of extracellular matrix (ECM) around cardiomyocytes and vascular cells, being recognized as a pathological entity of morbidity and mortality. Reducing reactive fibrosis is important for the prevention of excessive and inappropriate cardiac function, particularly after cardiomyocyte death due to cardiac injury and heart transplantation. Yet, preclinical studies on fibrosis treatment require human physiological approaches due to the multicellular crosstalk between cells and tissues in the heart. This study leveraged an induced pluripotent stem cell (iPSC)-derived multi-lineage human heart organoid (hHO) as a preclinical model to evaluate drugs for effective treatment reducing cardiac fibrosis and preventing progression of heart failure.

Cardiac fibroblasts (CFs) reactivation and differentiation into myofibroblast is the key process of cardiac fibrosis. TGF-β1 is the primary driver of myofibroblast activation in fibrosis. When this pathway is constantly active, it results in a continuous switch to express Collagen type I α1 (COL1A1), which is the main component of ECM deposits. This effect is finely controlled through interleukin-11 (IL-11) signaling. This study systematically explored cardiac fibroblasts activated upon TGF-β1 treatment and found activation of RAS pathway in cardiac fibroblast is intrinsically associated with COL1A1 production, particularly through its downstream axes, PI3K/AKT/mTORC1, Calcium signaling, and p38-MAPK pathways.

To explore RAS signaling activation in the context of cardiac fibrosis, this research investigated the inhibition of different downstream axes of RAS in activated CFs upon TGF-β1 stimulation. The PI3K/AKT/mTORC1 signaling was inhibited by Sirolimus and Calcium signaling was inhibited by Tacrolimus (TAC). Both inhibitors are also common immunosuppressants used in patients who received organ transplantation. p38-MAPK signaling was inhibited by SB202190, a selective inhibitor of p38α/β isoforms. In cardiac fibroblasts, SB202190 and Sirolimus reduced COL1A1 upon TGF-β1 stimulation, respectively. Next, the combination of SB202190 with the immunosuppressors was tested to see if it could foster the reduction of ECM production. Interestingly, the combined therapy of SB202190 with either TAC or Sirolimus had a profound impact on reducing COL1A1 expression.

However, experiments in hHO model showed this efficacy is reverted and worsened when TGF-β1 treated organoids are supplemented with SB202190 together with immunosuppressors. The sole usage of fibroblast cell line to study cardiac fibrosis could foster a bias of an oversimplified model that excludes fibroblast heterogeneity and the multicellular nature of human heart. This study addressed this problem by studying cardiac signaling in an iPSC-derived hHO model composed of organoid derived cardiac fibroblasts, cardiomyocytes, endothelial cells, endocardial cells and epicardial cells. In hHO, whereas Sirolimus showed reduced concentrations of COL1A1 in CFs, this phenotype is lost, leaving SB202190 as the only compound that effectively reduced TGF-β1 driven COL1A1 expression. The antifibrotic efficacy of combo treatment were also tested in hHO model and found massive ECM depositions upon pathological stimulation with TGF-β1. The SB202190 antifibrotic effects were reverted by the concomitant treatment with the immunosuppressors TAC and Sirolimus. IL-11 levels were rescued, and a severe fibrotic reactivation was found correlative to restoration in phosphorylated levels of p38, suggesting that immunosuppressants might influence non-fibroblast populations and secretory phenotypes contributed to non-cell-autonomous effects in cardiac fibrosis.

The human organoid platform enables multicellular crosstalk between cells in the heart, solving the bias to study cardiac fibroblast in isolation. This study reports a reliable preclinical model for evaluating drug cardiotoxicity and assessing cardiac fibrosis phenotypes in 3D hHOs derived from human iPSCs, endorsing their preclinical value, and exploring imperative roles of the heart microenvironment in response to treatments, opening new questions and highlighting the importance of the development of precision medicine in cardiovascular research.

（論文審査の結果の要旨）

心臓線維化は、組織損傷に対する線維芽細胞由来の適応プロセスが正常に働かず、心筋細胞や血管細胞の周囲に大量の細胞外マトリックス（ECM） が沈着する、罹患率と死亡率の高い病態として認識されている。本研究では、TGF-β1 処理によって活性化される心臓線維芽細胞を系統的に解析し、心線維芽細胞における RAS 経路の活性化が、特にその下流シグナル経路である PI3K/AKT/mTORC1、カルシウムシグナル、および p38-MAPK 経路を介して、ECM 沈着物の主成分であるⅠ型コラーゲンα1 （COL1A1） の発現調節に関与することを見出した。しかし、心臓線維化の治療に関する前臨床研究では、心臓の細胞や組織間の多細胞間クロストークに対応するヒト生理学的アプローチが必要である。そこで、本研究では、複数種の心臓細胞から構成されるヒト iPS 細胞由来の心臓オルガノイドを活用して、薬剤の心毒性を評価し、心臓線維化の表現型を評価するための前臨床モデルの基礎を築いた。心臓オルガノイドにおいて p38-MAPK 経路の阻害は COL1A1 の発現を有意に減少させるが、臓器移植に使用される免疫抑制剤であるタクロリムスまたはシロリムス（RAS の下流経路も阻害する）との併用治療はこの効果を打ち消した。以上の研究は心臓線維化における細胞間相互作用の存在の可能性を示唆し、臓器移植後の線維化抑制療法の開発に寄与するところが多い。

したがって、本論文は博士（　医　学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和 6 年 1 月 29 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降