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研究課題名（和文）Human induced pluripotent stem cell derived cardiomyocyte maturation by DNA integrative free-delivery of key regulators

研究課題名（英文）Human induced pluripotent stem cell derived cardiomyocyte maturation by DNA integrative free-delivery of key regulators

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：研究報告書において、私たちはhiPSC人工多能性幹細胞 由来の心筋細胞の成熟を安全に促進するための手法の開発に従事しました。これは再生医学における臨床応用の向上を目指すものです。最終的に、臨床応用に応用可能な、成人様の特性を備えたhiPSC-CMの増加を促す方法を開発しました。

研究成果の学術的意義や社会的意義
We developed methods with clinical applications to increase the maturation of cardiac tissues derived from human iPS exploiting the control of the cardiac cell cycle.

研究成果の概要（英文）：We extensively worked in developing safe methods to increase the maturation of hiPSC-derived cardiomyocytes to improve clinical applications in regenerative medicine.

Eventually, we developed methods to increase the adult-like properties of hiPSC-CM that can be transferred to clinical applications.

研究分野：Stem Cell Research in Cardiovascular Biology

キーワード：Cardiac maturation　TNNI3　hiPSC-CM　Stem Cell Research　Cell Cycle

１．研究開始当初の背景

Cardiomyocytes derived from human pluripotent stem cells (hiPSC-CMs) hold great potential for various applications in cardiovascular research. They can be utilized to investigate the developmental processes of cardiac tissue, including cardiogenesis, maturation, homeostasis, and aging. Additionally, hiPSC-CMs are valuable for disease modeling, assessing drug cardiototoxicity and understanding disease mechanisms, but most importantly, they serve as a cell source for regenerating functional cardiac tissue. This is particularly important considering the limited regenerative capacity of the human heart following injury, where hiPSC-CMs hold excellent potential for restoring myocardial function.

However, a major hurdle in utilizing hiPSC-CMs is their limited maturation compared to adult cardiomyocytes. Despite attempts to enhance maturation through cellular interventions, the fetal isoform of cardiac troponin I (TNNI1), which is indicative of an immature state, remains present in these cells. This suggests that current strategies primarily achieve neonatal maturation stages, rendering hiPSC-CMs potentially unsuitable for human functional transplantation. Consequently, the functional applicability of hiPSC-CMs is restricted.

Another challenge lies in the methods used to promote maturation, as many protocols and maturation assays involve DNA-integrative cellular interventions. These interventions compromise the long-term integrity and safety of hiPSC-CMs in clinical settings.

２．研究の目的

The objective of this study was to identify safe and clinically applicable methods for generating adult-like human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) in vitro using free DNA-integrative systems.

３．研究の方法

The methods employed in this study primarily involved the differentiation of ventricular cardiomyocytes from induced pluripotent stem cells. This was achieved by forming embryoid bodies (EBs) and subsequently seeding the cells onto a monolayer on Day 16. Once the cells exhibited beating activity, they were subjected to a 7-day treatment with specific compounds. To monitor the developmental switch in cardiac maturation between the fetal and adult isoforms of cardiac troponin I (TNNI1 to TNNI3), we utilized a reporter cell line. As a reference point for maturation, we treated hiPSC-CMs with Torin1, an mTOR inhibitor known to induce maturation and increase the expression of TNNI3 through cell quiescence. Following Torin1 treatment, additional small molecules were administered to reduce TNNI1 levels.

Functional assays were then conducted to assess maturation progress, including measurements of calcium handling, conduction velocity, sarcomere structure, and the metabolic switch to fatty acid oxidation (FAO).

４．研究成果

Inhibition of the mTOR pathway enhances the maturation of hiPSC-CMs. The pan-mTOR inhibitor, Torin1 was reported to partially contribute to the maturation of the myocardium through the activation of a p53-dependant quiescence. However, mTOR inhibition is insufficient to promote a complete developmental maturation of the functional myocardium beyond the neonatal period, which is featured by a complete molecular switch between TNNI1 and TNNI3 proteins. We suspected that this occur through a failure in reducing the levels of phospho-AKT, an important protein in the PI3K pathway involved in cellular growth, proliferation and a wider broad of effects opposing to cellular quiescence. We systematically explored the effects of small molecules and identified CX49 as able to continue the ectopic maturation of hiPSC-CMs *in vitro* by

reducing levels of TNNI1 while maintaining high levels of TNNI3 (Figure 1a)

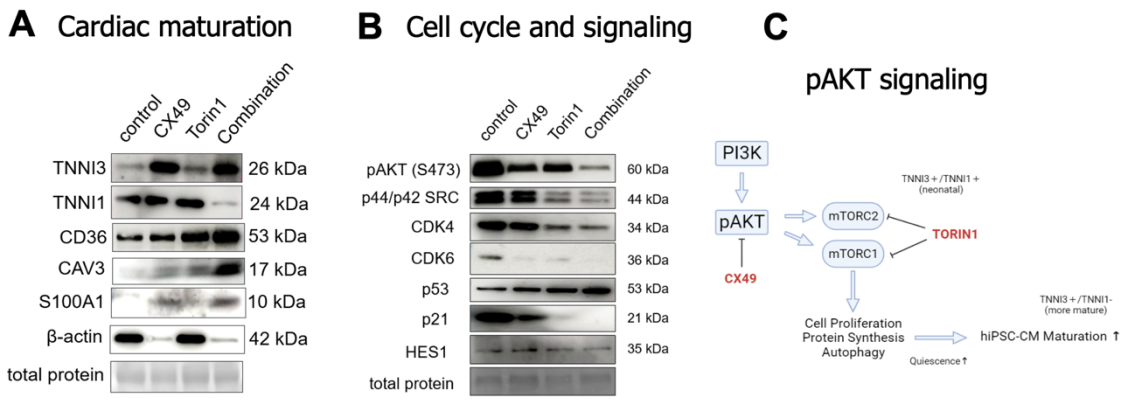

Figure 1. Characterization of cardiac maturation protein in hiPSC-CMs

The combination treatment demonstrates a notable pattern of significantly high expression of TNNI3 and low expression of TNNI1.(Figure 1A)

In terms of cardiac maturation, the combination treatment exhibits a notable expression of markers related to metabolic maturation (CD36), T-tubule formation (CAV3), calcium handling (S100A1), and cytoskeleton organization (β-actin). (Figure 1A)

Regarding cell cycle and signaling, the combination treatment strongly suppresses pAKT and pSRC. Additionally, it effectively inhibits CDK4/6 while exhibiting high levels of p53 and low levels of p21. These findings indicate that the combination treatment induces cellular quiescence. (Figure 1B-C)

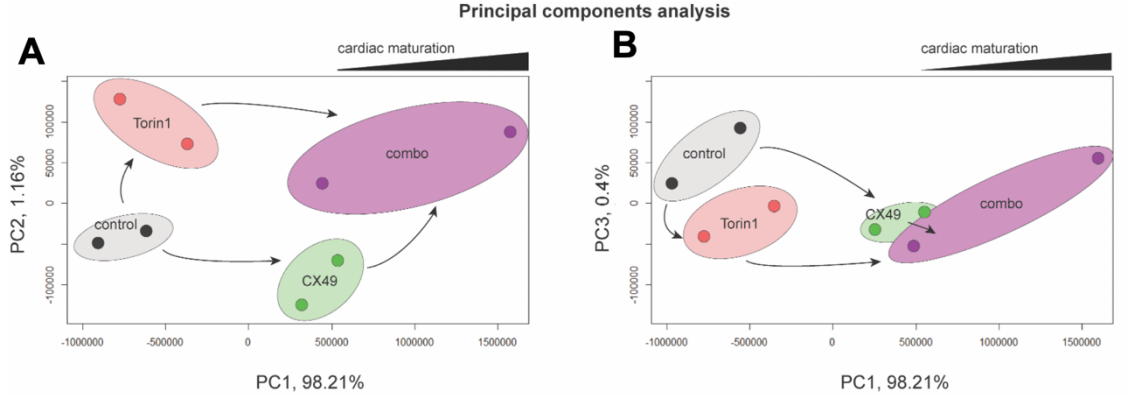

Figure 2. Principal component analysis of treated hiPSC-CMs

RNA-Seq analysis reveals that over 98% of the transcriptomic differences are explained through the principal component 1 (PC1), showing that the combo treatment has a profound impact on the cardiomyocyte transcriptomics (Figure 2A, 2B)

After achieving cardiomyocyte maturation through quiescence, the synergistic pathway enhances cardiac maturation, resulting in cardiomyocytes resembling adult cells' characteristics.

We also generated mature engineered heart tissue (EHT) (Figure 3A), showing that the combo treatment improved contractile force (Figure 3B), another important hallmark of cardiac maturation. This improvement is attributed to increased TNNI3 expression and decreased TNNI1 expression, which, in combination with other sarcomere-related proteins, contributes to the enhancement of cardiac function, particularly of the functional ventricle.

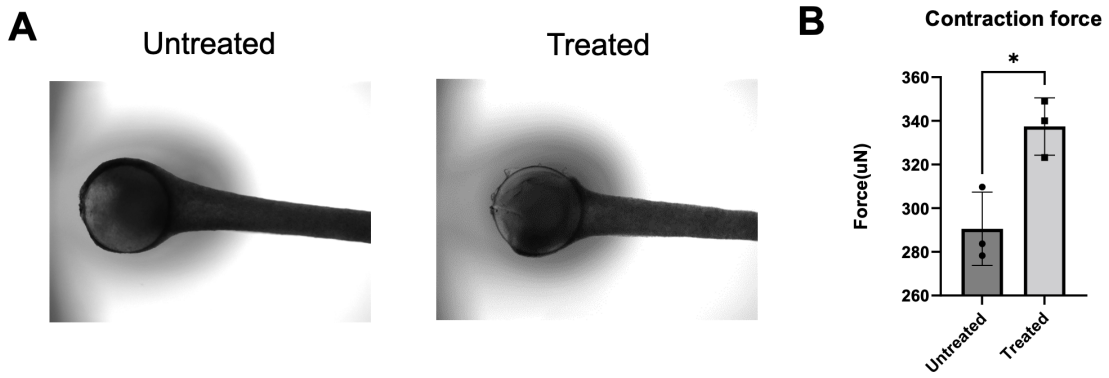

Figure 3. Generation of mature engineered heart tissue (EHT)

In summary, our study demonstrated that targeting the residual activity of pAKT in quiescent cardiomyocytes using small molecules resulted in an enhanced level of maturation in hiPSC-derived cardiomyocytes and engineered heart tissue (EHT). Furthermore, the combination treatment with Torin1 showed improvements in cardiac maturation, including structural, metabolic, and contractile aspects.

In conclusion, we successfully developed a free DNA-integrative system that effectively induces the maturation of hiPSC-CMs, bringing them closer to an adult-like state. The use of small molecules with an FDA-approved profile ensures the potential clinical applicability of these mature cardiomyocytes and EHT for therapeutic purposes.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　0件／うち国際共著　2件／うちオープンアクセス　0件）

１．著者名 Antonio Lucena-Cacace, Yoshinori Yoshida	４．巻 2320
２．論文標題 Analysis of Transcriptional Profiling of Chamber-Specific Human Cardiac Myocytes Derived from Pluripotent Stem Cells	５．発行年 2021年
３．雑誌名 Methods in Molecular Biology	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/978-1-0716-1484-6_20	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Julia Junghof, Yuta Kogure, Yu Tian, Eva Maria Verdugo-Sivianes, Megumi Narita, Antonio Lucena-Cacace, Yoshinori Yoshida	４．巻 7
２．論文標題 CDH18 is a fetal epicardial biomarker regulating differentiation towards vascular smooth muscle cells	５．発行年 2022年
３．雑誌名 Npj Regenerative Medicine	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41536-022-00207-w	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計0件

〔図書〕　計0件

〔出願〕　計0件

〔取得〕　計1件

産業財産権の名称	発明者	権利者
Mature-cardiomyocyte production method	Antonio Lucena-Cacace, etc	同左
産業財産権の種類、番号	取得年	国内・外国の別
特許、PCT/JP2021/007466	2021年	外国

〔その他〕

CDH18 は胎児期の心外膜細胞の指標であり胎児心外膜から平滑筋細胞の分化を制御している
https://www.cira.kyoto-u.ac.jp/j/pressrelease/news/220209-150000.html
心外膜細胞のバイオマーカーとして CDH18 を同定-CiRA ほか
http://www.qlifepro.com/news/20220214/epicardial-biomarker.html

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関