論　文　内　容　の　要　旨
Synopsis of Thesis

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論文題名 Title	Scaffold-Mediated Developmental Effects on Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes Are Preserved After External Support Removal (足場によるヒト人工多能性幹細胞由来心筋細胞の発達効果と持続可能性の検証)

論文内容の要旨

〔目　的(Purpose)〕

Human induced pluripotent stem (hiPS) cells have been used as a cell source for regenerative therapy and disease modeling. The purity of hiPS-cardiomyocytes (hiPS-CMs) has markedly improved with advancements in cell culture and differentiation protocols. However, the morphological features and molecular properties of the relatively immature cells are still unclear, which has hampered their clinical application. The present study aimed to investigate the extent to which topographic substrates actively influence hiPS-CMs.

〔方法ならびに成績(Methods/Results)〕

Anisotropic patterned culture techniques (aligned patterning) have been extensively used to induce hiPS-CMs maturation. However, their efficiency has been varying among previous studies, probably due to technical differences, such as patterning materials and/or culture protocols. Therefore, firstly, to confirm if the aligned cell arrangement can induce maturation of iPS-CMs, hiPS-CMs were seeded on randomized oriented fiber substrate (random), anisotropic aligned fiber substrate (align), and flat non-scaffold substrate (flat). After culturing for one week, the hiPS-CMs on the aligned patterns showed more mature-like properties, including elongated rod shape, shorter duration of action potential, accelerated conduction velocity, and elevated cardiac gene expression.

Subsequently, to investigated the memory of iPS-CMs for the aligned pattern-induced facilitation of maturity, the cells were harvested from the different substrates and were then replated on the non-scaffold (flat) pattern for an additional week. As a result, the improvements in morphological and functional properties diminished, however, the hiPS-CMs pre-cultured on the aligned pattern somehow retained the memory of the beneficial effects from topographic induced maturation and maintained during development even without the fiber matrix.

To obtain insights into the underlying molecular pathways/signaling involved in the facilitation of hiPS-CM maturation driven by specific topographic stimuli, we performed RNA-seq on hiPS-CM samples after different patterns culturing. The RNA-seq data revealed that several cardiac maturation markers, such as MYH7 and TNNI3, were significantly upregulated in the align group, which is consistent with the qPCR results. Upon cross analysis of the upregulated genes in align group compared to the flat or random group, six genes were found to be specifically upregulated in the align pattern (including F13A1, XIRP2, TTC29, PTPRH, TULP1, and LINC00702). Based on our findings, the aligned topographic cell arrangement may be related to the regulation of CM maturation via modulation of the cell cycle.

〔総　括(Conclusion)〕

Our results suggested that the anisotropic fiber substrate can induce the formation of geometrical mimic-oriented heart tissue in a short time. Although the morphological and electrophysiological properties of hiPS-CMs obtained via facilitated maturation somehow rely on the existence of an exterior scaffold, the molecular developmental features were preserved even in the absence of external support, which might persist throughout hiPS-CM development.

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

ヒト人工多能性幹（hiPS）細胞は、再生治療や疾患モデリングのセルソースとして使用されているが、成熟細胞組織への分化に伴う形態学的特徴と分子特性は未だ不明であるため、臨床応用の問題点になっている。本研究は、培養足場形態によるヒト人工多能性幹細胞由来心筋細胞（hiPS-CM）の発達効果と持続可能性の検証を目的とした。hiPS-CMは、配向性を持つ足場で培養することにより、形態学的および電気生理学的に外部足場の存在に依存して成熟心筋組織と近い特性を示した。心筋の成熟を示す遺伝子レベルの分子特性は足場から剥離し外部サポートから解放された場合でも保持されることが明らかになった。

本研究は、hiPS-CM成熟の過程において有効な足場形態がネイティブハートと近い成熟した心臓組織の形成を誘導する事を示唆し、心臓再生医療分野における基礎研究と臨床応用の両方において重要な知見を提供した。従って本研究は博士（医学）の学位を授与するに値する。