Project Title:	Development of Novel Method to Generate Mature Cardiomyocytes Derived from Human Pluripotent Stem Cells 開發誘導血管再生的策略
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The capacity of hPSC-CMs provides an unlimited resource for human CMs for various applications such as cell-based cardiac repair, cardiac drug toxicology screening, and human cardiac disease modeling. However, applicability is significantly limited by their immature phenotypes. Several new approaches to generate more mature hPSC-CM have been developed to overcome this critical issue. However, methodologies for maturing hPSC-CMs are rapidly improving; even these improved methods only result in heterogeneous populations of hPSC-CMs of varying maturities. Thus, developing a method that can selectively purify mature hPSC-CMs is necessary.

In this grant, the team aimed to develop a novel system that can purify homogeneous populations of mature hPSC-CMs via the antibody targeting CD36 surface protein markers uniquely expressed in mature CMs. The maturation of hPSC-CMs in vitro was promoted by using two independent methods, all of which were previously reported. Briefly, the team produced 3D fibrin-based cardiac tissues with day 15 (d15) hPSC-CMs and continuously cultured them until day 45 (d45). In addition, an extended culture model was generated by continuously culturing 15 hPSC-CMs up to day 100 (d100). To enrich functionally mature hPSC-CMs, the hPSC-CMs dissociated from the cultured 3D cardiac tissues or the 100-day-cultured hPSC-CMs were treated with CD36 Ab, fluorescence-activated cell sorting (FACS)-sorted, and then subjected to various assays such as qRT-PCR, immunocytochemistry, and several functional/physiological examination including Ca^{2+} transient and patch clamp analysis. After performing several assays examining CM identity and functional maturity, it was found that these enriched CD36+ hPSC-CMs were more mature than control hPSC-CMs. To check the functionality of FACS-sorted CD36-positive hPSC-CMs (CD36+ hPSC-CMs), the team performed three analyses: 1) Ca^{2+} transient, 2) patch clamp and, 3) MEA analysis, all of which are well-known assay for evaluating the physiological function of the cardiomyocyte.

As a result, in the Ca^{2+} transient analysis, all three types of CMs showed automaticity, but the patterns of calcium transients of CD36+ hPSC-CMs were similar to mature ventricular CMs but not atrial CMs; the frequency was slower, and the amplitude was larger in CD36+ hPSC-CMs, indicating that these cells are likely to mature ventricular type CMs. In addition, the team obtained stable recordings from forty-six purified CD36+ hPSC-CMs in the patch clamp analysis. The results provide the first evidence for identifying the novel surface markers that distinguish mature CMs from a pool of immature CMs.