Sarcomere disassembly and transfection efficiency in proliferating human iPSC-derived cardiomyocytes

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Introduction: Proper contraction of cardiomyocytes (CMs) is dictated by the architecture of the sarcomeres and appears together with polyploidy inversely correlated with limited self-renewal and regeneration of the adult heart. This limited regenerative potential correlates with the decrease in mononuclear diploid cells in adult versus neonatal myocardium. Previous animal studies have shown that the sarcomeres are disassembled during proliferation of neonatal CMs. However, little is known about sarcomere assemblage during human CMs nuclear and/or cell division, which would be relevant information for strategies to boost endogenous heart repair.

Purpose: In this study we used the human induced pluripotent stem cell (hiPSC) model to investigate sarcomere assemblage during mitosis, followed by cytokinesis, multinucleation and/or self-duplication in massively expanding hiPSC-CMs. Furthermore, we examined whether CMs undergoing nuclear and/or cellular duplication would be more susceptible for genomic editing than non-proliferative hiPSC-CMs.

Methods: hiPSC-CMs were massively expanded using our previously described CHIR99021 small molecular treatment and cell-cell contact removal. We then performed immunofluorescence staining with cardiac Troponin T (cTnT) and Ki67 to follow sarcomere organization during cell cycle activity. Next, we performed Time-Lapse imaging in a hiPSC-CM culture system to follow the sequence of cellular and nuclear divisions in hiPSC-CMs. RNA sequencing lookup was performed for proliferation, sarcomere and molecular pathway-related genes. Efficiency of non-viral vector-based fluorescence (mCherry) expression and transfection efficiency was done by flow cytometry analysis and Time-Lapse imaging.

Results: We observed that both mononuclear and binuclear hiPSC-CMs give rise to mononuclear daughter cells or binuclear progeny. Within this source of highly proliferative hiPSC-CMs, treated with CHIR99021 small molecule, we found that Wnt and Hippo signaling were more present when compared to metabolic matured and non-proliferative hiPSC-CMs and adult human heart tissue. Moreover, we found that CHIR99021 treatment increased the efficiency of non-viral vector incorporation in high-proliferative hiPSC-CMs, in which fluorescent transgene expression became present after completion of chromosomal segregation (M-phase).

Conclusion: This study demonstrates that there is a complex landscape in proliferation, multinucleation and self-duplication of human CMs. Moreover, enhanced incorporation of alien non-viral vectors is related to Wnt activation and cell cycle activity of hiPSC-CMs. Altogether, our findings provide insights in duplication of human CMs and tools for molecular gene studies.