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学位論文題目	THE ROLE OF CELL CYCLE IN DIRECT REPROGRAMMING OF INDUCED CARDIOMYOCYTES （心筋への直接転換における細胞周期の機能解析）

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論文の要旨
Abstract of thesis

1. PURPOSE:

Direct reprogramming of fibroblasts into induced cardiomyocytes (iCMs) holds great promise for cardiac regenerative medicine. Several mechanisms of iCM-reprogramming have been studied so far; however, cell-cycle regulation during iCM-reprogramming has not. The applicant aimed to understand cell-cycle regulation of reprogrammed iCMs and to investigate how cell-cycle manipulation affects iCM-reprogramming.

2. METHODS & RESULTS:

For iCM-reprogramming, αMHC-GFP mouse embryonic fibroblasts (MEFs) were infected with three monocistronic retroviruses of Gata4, Mef2c, and Tbx5 (GMT). The time-lapse recordings at day 2 post-infection (DPI-2) demonstrated that MEFs were initially reprogrammed into αMHC-GFP+ iCMs with faint fluorescence (GFPlow) and then gradually gained more intense fluorescence (GFPhigh). EdU proliferation assays showed that GFPhigh iCMs contained much less EdU+ cells and expressed many cardiac genes significantly higher than GFPlow cells, demonstrating a relatively more advanced reprogramming of GFPhigh iCMs. The applicant found by time-lapse recordings that nearly half of GMT-iCMs divided within 48 hours after the activation of αMHC-GFP and showed by EdU proliferation assays that iCMs exited cell cycle along the

process of reprogramming with a decreased percentage of EdU+/αMHC-GFP+ cells. S-phase synchronization by Aphidicoline, Hydroxyurea, and L-mimosine for 24 hrs at DPI-1 significantly enhanced cell-cycle exit of GMT-iCMs. Noticeably, compared to monocistronic GMT, polycistronic MGT (Mef2c-P2A-Gata4-T2A-Tbx5) facilitated cell-cycle exit and yielded significantly higher portion of GFPhigh iCMs. Importantly, S-phase synchronization increased the yield of GFPhigh iCMs reprogrammed by GMT, but failed to further accelerate the progression of MGT-reprogramming, suggesting that both S-phase synchronization and MGT accelerated reprogramming through enhanced cell cycle exit.

In summary, iCMs were induced majorly at late-G1 and S phases, did go through cell division at the early stage of reprogramming, and ultimately exited cell cycle at G0/G1 phase during the process of reprogramming. Importantly, S-phase synchronization at the critical reprogramming-initiation time (DPI-1) or polycistronic MGT facilitated the early progression of GMT-reprogramming and yielded more GFPhigh iCMs, which are relatively higher quality iCMs among the reprogrammed cells, through enhancing cell-cycle exit.

3. DISCUSSION & FUTURE DIRECTION:

The facilitated cell-cycle exit by S-phase synchronization at DPI-1 was accompanied with an improved progression of GMT-reprogramming and yielded significantly more GFPhigh iCMs, which achieved a more advanced reprogramming than GFPlow cells. This might be due to that cell-cycle exit prevents a dilution of GMT expression in individual iCMs and subsequently induce high cardiac gene expression and better reprogramming. It is also true in iCM-reprogramming of polycistronic MGT, which accelerated cell-cycle exit and yielded more GFPhigh iCMs. Because of this accelerated progression of MGT-reprogramming, S-phase synchronization failed to further increase the GFPhigh portion in MGT-iCMs. It has been shown that active cell-cycle status and maturity have negative correlation in iCMs. On the other hand, it has been observed that iCM-reprogramming was significantly suppressed in an immortalized cardiac fibroblast line that will never exit cell cycle, indicating the importance of cell-cycle exit in iCM-reprogramming. All these mark the importance of maintenance of cell cycle activation at the early stage of reprogramming and the necessity of cell-cycle exit on maturation of iCMs as possibly similar to process of maturation in native mammalian cardiomyocytes. Recently, it has been demonstrated that cell-cycle inactive fibroblasts failed to be reprogrammed, suggesting that cell-cycle activation could be necessary for initiation and early progression of iCM-reprogramming. Similarly, the applicant showed nearly half of iCMs at early stage of reprogramming divided. On the other hand, this study revealed the impact of induced cell-cycle exit on iCM maturation. In vivo reprogramming constitutes the most important part of the cardiac reprogramming technology; however, it is still not translatable to real-life cases as cardiac fibroblasts in the chronic type of ischemic heart disease are quiescent (cell-cycle inactive). Collectively, the findings by others and the applicant indicate that the quiescent cardiac fibroblasts in fibrotic scar in

vivo might need to be cell-cycle activated prior to initiation of reprogramming, which in turn could increase the number of in vivo iCMs and improve heart function better. Therefore, novel methods should be generated for cell-cycle activation of quiescent cardiac fibroblasts for in vivo reprogramming. Moreover, rather than acute myocardial-infarction in animal models, chronic models of heart diseases should be employed in cardiac reprogramming studies as to mimic regeneration of damaged heart in real disease situations in human patients.

審査の要旨
Abstract of assessment result

【批評 Review】
The applicant hypothesis is that direct reprogramming efficiency into iCMs is influenced by cell cycle. He tested carefully his hypothesis by a series of experiments and confirmed that precedent cell cycle synchronization and cell cycle exit are important for direct reprograming into cardiomycytes. His findings will help understanding molecular mechanism of direct reprogramming and efficiency for clinical use.

【最終試験の結果 Result】
The final examination committee conducted a meeting as a final examination on 25 December, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】
Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.