指導教授氏名 Instructing Professor Commissioned Professor	指導役割 Role
（自署）	Supervisor and Research Guidance
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学位論文要旨
Abstract of the Doctoral Dissertation

岡山大学大学院医歯薬学総合研究科

教育研究分野 Department	Oral Rehabilitation and Regenerative Medicine	身分 大学院生	氏名 Name	DO Thuy Hang
論文題名 Title of Doctoral Dissert	Retrospective re-discovery of the transcription factor that controls chondrocyte differentiation by single cell RNA-sequencing (一細胞解析を応用した軟骨細胞分化を制御する転写因子の遡及的な再発見法)			

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[Introduction]

The intricate process of differentiation from stem cells to somatic cells involves a dynamic transition of the transcriptome, where transcription factors are crucial. Among these, SRY-box transcription factor 9 (*SOX9*) is recognized as a key regulator of chondrogenesis, but the factor inducing *SOX9* along the differentiation pathway remains unclear, partly due to challenges in characterizing transcriptomic transitions *in vivo*.

In contrast, somatic cell reprogramming into induced pluripotent stem cells (iPSCs) seems straightforward, primarily involving the overexpression of four Yamanaka factors. However, this process may not be uniform across all cells, suggesting cell-type-specific mechanisms. Recent studies suggest that reprogramming follows a pathway resembling early differentiation, with the activation of certain retroviruses playing a critical role.

A hypothesis proposes that somatic cells revert to iPSCs by retracing their natural differentiation pathway backward. To test this, we analyzed the transcriptomic trajectory from chondrocytes to iPSCs using single-cell analysis. This study revealed the potential of inverse genetics in identifying master regulators of cell differentiation. Unexpectedly, off-targeted cells followed a distinct pathway.

[Methods]

To address these challenges, we employed a dual approach combining genetic reprogramming with advanced single-cell RNA sequencing (scRNA-seq) to investigate the transcriptomic changes that occur when human articular chondrocytes are reprogrammed into induced pluripotent stem cells (iPSCs). The reprogramming was facilitated using an RNA virus (SRV iPSC-3 vector), which delivered key pluripotency factors including *OCT4*, *SOX2*, *KLF4* and *c-MYC*.

Following the introduction of the viral vectors, the chondrocytes were cultured under defined conditions that promote iPSC formation. Cells were harvested at daily time points during the reprogramming process and subjected to scRNA-seq using the 10X Genomics platform. This high-throughput method allowed us to

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capture a detailed snapshot of gene expression patterns across thousands of individual cells, facilitating the identification of distinct cellular states and trajectories during the reprogramming process.
To verify that chondrocytes go back to pluripotency following the proper differentiation route in an inversed fashion, iPS-interference technique (Hikichi et al., PNAS, 2013) was also employed.

[Results]

Analysis of the scRNA-seq data revealed two primary transcriptomic pathways emerging during the reprogramming of chondrocytes to iPSCs. The first pathway was characterized by successful reprogramming, where cells showed a significant upregulation of pluripotency markers such as *NANOG* and *SOX2*, coupled with the critical downregulation of *SOX9*. This pathway confirms the importance of suppressing *SOX9* early in the reprogramming process to facilitate the transition to a pluripotent state.

The second pathway highlighted cells that retained a chondrocyte identity, evidenced by persistent expression of chondrocyte markers such as *PRG4* and *SOX9*. These cells failed to be reprogrammed successfully into iPSCs, suggesting that incomplete silencing of *SOX9* may prevent the full transition to pluripotency. This finding underscores the role of *SOX9* not only as a marker of chondrocyte identity but also as a potential barrier to reprogramming if not adequately suppressed.

Furthermore, iPS interference strategy was employed to confirm that chondrocytes were successfully converted into pluripotent cell colonies, with the crucial step being the turning off of *SOX9* expression during the reprogramming pathway. Colonies with typical iPSC-like morphology and positive staining for human embryonic stem cell-specific markers (rBC2LCN) indicated successful reprogramming, while overexpression of *SOX9* hindered this process, highlighting its role in directing mesenchymal stem cells towards chondrogenic differentiation.

[Conclusions]

Our study illuminates the complex dynamics of cell identity during the reprogramming of chondrocytes to iPSCs, highlighting the pivotal role of the transcription factor *SOX9*. The findings demonstrate that *SOX9* must be effectively silenced to achieve successful reprogramming, providing critical insights into the mechanisms of cellular reprogramming and pluripotency. Further investigations based on these findings could lead to novel approaches for manipulating *SOX9* and other key transcription factors to enhance the therapeutic potential of stem cell technologies. These advances hold promise not only for treating cartilage injuries but also for addressing a broader range of degenerative conditions where effective cell differentiation and reprogramming are crucial.