学位論文抄録

Replacement of Sox2 in reprogramming by identification of the biphasic role of calcineurin pathway

（二方向性のカルシニューリン経路による Sox2 非依存的リプログラミングの同定）

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Abstract of the Thesis

Background and Purpose: Somatic cell reprogramming using defined factors (Oct4, Sox2, Klf4 and c-Myc) known as OSKM to produce induced pluripotent stem cell (iPS cell) raised new hopes for successful clinical trials of stem cell therapy. Despite that, it would be crucial to understand the detailed molecular and epigenetic modifications for ensuring the safety of their application. Here, we aimed in our study to dissect the role of calcineurin and their downstream NFAT targets in reprogramming.

Methods: To study the role of calcineurin/NFAT pathway, we used chemical and genetic inhibitors to inhibit the calcineurin activity and their downstream targets NFATc isforms during each phase of reprogramming. We studied the accompanied change at the level of RNA expression by qPCR, histone modifications by ChIP-qPCR, protein level by western blot, cytoplasmic/nuclear translocation by immunostaining, enzymatic activity by ELISA, protein-protein interaction by immunoprecipitation, cell cycle analysis by flow cytometry , estimation reprogramming efficiency by alkaline phosphatase staining, overexpression and knockdown by retroviral and lentiviral infections.

Results: Calcineurin exhibits a dual opposing role in reprogramming. In the early phase, calcineurin in required for maintaining proper cell cycle proliferation, and knockdown of calcineurin results in arresting cells in G1 phase with delaying in mesenchymal and epithelial transition (MET) rate. In the late phase, calcineurin possesses a negative role mediated by transiently expressed NFATc2, which translocates to the nucleus where it recruits Suv39h1, hdac3 and ezh2 over Sox2 and Klf2 loci and repress their expression by increasing the relative enrichment of the repressive mark H3k9me3 resulting in decreasing reprogramming efficiency. We also identified Gnaq as an upstream regulator of calcineurin. By knockdown of calcineurin, we can replace Sox2 in reprogramming process.

Conclusions: Overall results show that calcineurin can regulate the reprogramming in stage specific manner, and by critically analyzing the calcineurin and its downstream targets, we can replace Sox2 in reprogramming process. We also clarified the new regulatory Gnaq/calcineurin/NFATc2 loop, which could be useful for biological studies.