A lineage study for cardiac conduction system in mice

Sakaguchi, Akane

Doctor of Philosophy

Department of Genetics
School of Life Science
SOKENDAI (The Graduate University for Advanced Studies)

A lineage study for cardiac conduction system in mice

20101851 Sakaguchi, Akane

Department of Genetics

School of Life Science

SOKENDAI

(The Graduate University for Advanced Studies)

Introduction

The cardiac conduction system (CCS) is the cellular network system to generate the electric stimulation for the constant heart beating. It is composed of special cardiomyocytes called pacemaker cells and Purkinje cells. Pacemaker cells in the sinoatrial node (SAN) which exists on the posterior wall of the right atrium (RA) near the entrance of the superior vena cava (SVC) generated electric stimulation autonomously, then the stimulation spreads to the atrium to induce atrial contraction and converged atrioventricular node (AVN).The AVN is also composed of pacemaker cells that propagates the electric pulse to ventricles through Purkinje fibers for separating the contractions of atrial and ventricular chambers. The defects in the CCS induce not only an irregular beating rhythm but also insufficient electric stimulation for contraction, which leads the cardiac dysfunction called cardiac arrhythmia. This disease occurs by both congenital and acquired reasons, and is known as one of the causes of sudden death. Although the CCS is composed of special cardiomyocytes, the cellular origin and developmental process are different from those of other cardiac components. It was reported that almost all cardiac components are derived from cells transiently expressing the transcription factor Mesp1, which is the master regulator of cardiovascular development at embryonic day 6.5 (E6.5) in mice. However, the descendants of *Mesp1* is not contribute to the CCS. On the other hand, previous cell tracking analyses indicated that CCS progenitors are derived from mesodermal cells at E7.5. These cells are located the caudal-lateral side of the cardiac primordia called cardiac crescent, and the cell is not expressed *Nkx2.5*, which is known as a marker gene for cardiac crescent. On the other hand, the cell tracking analyses indicate that the progenitor cell for CCS exist on the posterior side of the cardiac crescent. The developmental process of the chick embryo is

similar to the murine, however, the cell tracking analysis cannot be conducted in the mouse because of the technical problem. These reports suggest that CCS progenitor cells exist at E7.5, however, the cell has not been identified because of the lack of appropriate marker gene.

Results

In order to identify the CCS progenitor cell, first I focused on the *Secreted frizzled related protein 5* (*Sfrp5*). Our laboratory revealed that the *Sfrp5* expressing cell at E7.5 is a cardiac progenitor cell contributing to sinus venosus primordia (SVP), which is transiently formed containing some progenitor cells including CCS progenitor cell. However it has not been studied whether *Sfrp5* lineage from E7.5 contribute to the CCS with marker expression. I generated KI mice which CreERT2 is knocked-in into the *Sfrp5* locus, and traced the lineage from the cardiac crescent stage to the CCS progenitor (E10.5) and matured CCS stages (E16.5) with the tamoxifen treatment. The descendant from E7.5 was differentiated into the CCS and myocardium at E16.5, suggesting that *Sfrp5* is not a specific marker for CCS progenitor cells but *Sfrp5* expressing cells at E7.5 include the CCS progenitor cell. Therefore, next I focused on the difference of the contribution between *Mesp1* and *Sfrp5* lineages. In order to trace the cell lineage simultaneously in a single embryo, I generated Dre knocked-in mice into the *Mesp1* locus, and established *Mesp1*-Dre/*Sfrp5*-CreERT2 mice. Double lineage tracing analyses revealed that *Sfrp5* expressing cell, which is not derived from *Mesp1* lineage (*Mesp1*-negative/*Sfrp5*-expressing cell) at E7.5 contributed to the CCS progenitor cell at E10.5 and it differentiated into the CCS at E16.5. To observe these cells at E7.5, I crossed *Mesp1*-Cre mouse with CAG-floxed CAT-mCherry/*Sfrp5*-venusYFP KI mice, and found

that *Mesp1*-negative/*Sfrp5*-expressing cells were frequently observed on the posterior side of the cardiac crescent. It implies that the area is the candidate that includes CCS specific progenitor cells. To further restrict the CCS progenitors, I conducted lineage tracing analyses focusing on another mesodermal gene. Its lineage tracing analysis with *Mesp1*-lineage revealed that the contribution was observed in posterior part of the cardiac crescent and differentiate into subcomponents of the CCS, indicating that the CCS progenitor cell exist on the posterior part of the cardiac crescent and it established before the cardiac crescent stage.

Conclusion

Based on the double lineage tracing analyses using Cre-LoxP and Dre-Rox recombination system, I found that the posterior side of the cardiac crescent is the strong candidate for the CCS progenitor region. I expect that isolating the progenitor cells in this region should enable us to identify differentially expressed genes and help us to understand the developmental process of each CCS component.