ADGRL2 is an essential surface molecule for cardiac lineage specification and heart development

Authors:
HJ Cho¹, CS Lee¹, JW Lee¹, HM Yang¹, HS Kim¹, Seoul National University Hospital, Department of Internal Medicine - Seoul - Korea (Republic of),

Background: Specific surface markers that enable monitoring of cell subsets would be valuable for establishing the conditions under which pluripotent stem cells (PSCs) differentiate into cardiac progenitor cells (CPCs) and cardiomyocytes (CMCs).

Methods and Results: To verify whether a specific marker is expressed during heart development, we assessed its expression using the CLARITY technique. After immersion in a solution with a refractive index matching that of the CLARITY hybrid, the mouse embryo became transparent. After immunostaining the cleared embryo sample, Adgrl2 was exclusively observed in cardiac cells expressing a-SA at embryonic day E9.5 and E10.5. Our clarified 3D images and movies show that four chambers of the heart are fully developed at E10.5 but not at E9.5. At E9.5, Adgrl2 is observed at the ventricle and atrium, while Adgrl2 is present in all chambers of the heart at E10.5. Next, we performed LacZ (β-Gal) staining in heterozygous Adgrl2 KO embryos to evaluate Adgrl2 expression. As a result, LacZ staining showed that Adgrl2 was predominantly expressed in the heart during the embryonic developmental stage. Adgrl2 knockout in mice was embryonically lethal because of severe heart, but not vascular, defects. To examine the use of Adgrl2 as a bona fide CPC marker during heart development, we tracked Adgrl2 expression during early embryonic development. The heart of Adgrl2−/− embryos at E10.5 exhibited occlusion of the RV, and the expression levels of Gata4 and Nkx2.5 were not as high as those in wild-type and Adgrl2+/− embryos. Interestingly, the heart of Adgrl2−/− embryos, unlike those of wild-type and Adgrl2+/− embryos between E13.5 and E15.5 had a single ventricle revealing a ventricular septal defect. The specific expression pattern of Adgrl2 in PSC-derived cardiac lineage cells as well as in embryonic heart, adult mice, and human heart tissues.

Conclusion: We demonstrate that Adgrl2 plays a pivotal and functional role across all strata of the cardiomyogenic lineage, as early as the precursor stage of heart development. These findings shed light on heart development and regeneration.