Mesenchymal stem cells facilitate cardiac differentiation in Sox2-expressing cardiac C-kit cells in coculture

ABSTRACT

Stem cell therapy offers hope to reconstitute injured myocardium and salvage heart from failing. A recent approach using combinations of derived Cardiac-derived c-kit expressing cells (CCs) and mesenchymal stem cells (MSCs) in transplantation improved infarcted hearts with a greater functional outcome, but the effects of MSCs on CCs remain to be elucidated. We used a novel twostep protocol to clonogenically amplify colony forming c-kit expressing cells from 4- to 6-weekold C57BL/6N mice. This method yielded highly proliferative and clonogenic CCs with an average population doubling time of 17.2 ± 0.2, of which 80% were at the G1 phase. We identified two distinctly different CC populations based on its Sox2 expression, which was found to inversely related to their nkx2.5 and gata4 expression. To study CCs after MSC coculture, we developed micron-sized particles of iron oxide-based magnetic reisolation method to separate CCs from MSCs for subsequent analysis. Through validation using the sex and species mismatch CC-MSC coculture method, we confirmed that the purity of the reisolated cells was greater than 85%. In coculture experiment, we found that MSCs prominently enhanced Ctni and Mef2c expressions in Sox2 pos CCs after the induction of cardiac differentiation, and the level was higher than that of conditioned medium Sox2 pos CCs. However, these effects were not found in Sox2 neg CCs. Immunofluorescence labeling confirmed the presence of cardiac-like cells within Sox2 pos CCs after differentiation, identified by its cardiac troponin I and α-sarcomeric actinin expressions. In conclusion, this study shows that MSCs enhance CC differentiation toward cardiac myocytes. This enhancement is dependent on CC stemness state, which is determined by Sox2 expression.

Keyword: Sox2; Cardiac c-kit cells (CCs); Cardiac differentiation; Magnetic cell re-isolation; Mesenchymal stem cells (MSCs); Micron-sized particles of iron oxide (MPIO)