ABSTRACT

Cardiovascular Diseases (CVDs) with loss of functional cardiomyocytes and irreversible damage to heart, remain the major cause of worldwide mortality and morbidity. Recent advances in stem cell therapy field gives hope to restore normal heart function by rejuvenating damaged myocardium with new functional cardiomyocytes. Cardiosphere Derived Cells (CDCs) are a type of Cardiac Stem Cells (CSCs) that express stemness and early cardiac markers, including but not limited to c-kit, Sca-1, Isl-1, MDR 1, Nkx 2.5 and GATA 4. CDCs are advantageous in myocardial regeneration therapy because of their clonogenic, anti-inflammatory and immunomodulatory properties along with the paracrine activity by secretion of growth factors.

Development of an efficient and reliable protocol to direct CDCs from multipotent stage to cardiomyogenic state under in vitro conditions will provide an indispensable model to understand molecular mechanisms involved in differentiation. 5-Azacytidine (Aza), a known DNA methylation inhibitor and Ascorbic Acid (AA), a common co-factor in numerous biological reactions could influence self-renewal and differentiation of stem cells. Wnt/β-catenin signaling pathway is highly conserved in the organisms and regulates cellular functions like proliferation, differentiation and maintenance both during normal development and diseased conditions. The present study was designed to evaluate the role of Aza alone or Aza+AA in mediating CDCs to cardiomyogenic lineage and to understand the involvement of Wnt/β-catenin pathway in the same.

Rodent heart explant culture was performed and the initial Cardiac Explant Outgrowth Cells (CEOCS) were propagated as Cardiospheres (CS) in
suspension from which CDCs were expanded. Immunofluorescence (IF) analysis of CEOCs by Z-stack method displayed distinct expression of c-kit in the freshly shed outgrown cells from the explant. Whereas, IF analysis of CDCs revealed that c-kit expression was merely observed whereas CD 105 expression was prominent. Reverse transcriptase polymerase chain reaction analysis confirmed that CEOCs expressed stemness markers like c-kit and Isl-1 whereas CDCs expressed early cardiomyogenic markers, Nkx 2.5, GATA 4 and CD 90.

After optimization of Aza and AA concentrations, CDCs were treated with Aza alone or in combination with AA to differentiate CDCs to cardiomyocytes. Growth curve analysis revealed that proliferation was decreased in Aza treated CDCs when compared to Aza+AA treated CDCs. Colony Formation Unit-Fibroblasts assay (CFU-F) revealed that colony formation capacity was consistent in control CDCs than the treated groups. Gene and protein expression analysis proved that Aza+AA treated CDCs were high in expression of early cardiomyogenic markers like Nkx 2.5 and GATA 4 and cardiac structural protein, α-sarcomeric actinin. Wnt pathway was active in CDCs and the results indicated that Aza+AA down regulated the Wnt singaling pathway via β-catenin phosphorylation while directing CDCs to cardiomyogenic lineage.

Mitochondrial membrane potential and calcium imaging analysis revealed that membrane integrity was high with proper intracellular calcium signaling in Aza+AA treated CDCs. Observation of spontaneous beating of cells in Aza+AA treated CDCs further reinforced the ability of Aza+AA in mediating differentiation of CDCs. In conclusion, these outcomes at in vitro level suggest that stimulation of CDCs with Aza+AA treatment could prominently enhance proliferation and differentiation of CDCs with down regulation of Wnt/β-catenin pathway by phosphorylation of β-catenin.