INFLUENCE OF 3D CULTURE MICROENVIRONMENT ON MICRORNA-MEDIATED DIRECT REPROGRAMMING OF HUMAN CARDIAC FIBROBLASTS INTO CARDIOMYOCYTES

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Background

Direct reprogramming of cardiac fibroblasts into induced cardiomyocytes (iCMs) represents a new strategy to restore cardiac functions after myocardial infarction. Recently, a combination of four miRNAs (miR-1, 133, 208 and 499, named "miRcombo") has been reported to directly reprogram murine fibroblasts into iCMs. Furthermore, reprogramming efficiency was found increased when cells were cultured in a three-dimensional (3D) system. In this work, we analysed miRcombo-mediated reprogramming of human adult fibroblasts into iCMs embedded in a 3D hydrogel respect to 2D cultures.

Methods

AHCFs (Lonza, CC-2903) were transfected with miR-1, 133, 208 and 499 (mirVana) using DharmaFECT1 (Dharmacon). NegmiR (mirVana) transfected AHCFs were used as control. After 24 hours, transfection mix was removed. For 3D culture system, cells were detached and embedded in a fibrin-based hydrogel composed of 5 mg/mL fibrinogen (Merck) and 50 u/mL thrombin (Merck).

Results

After 4 days of culture, the expression of cardiac transcription factors (Gata4, Tbx5 and Nkx2.5) was enhanced in both miRcombo and negmiR transfected AHCFs cultured in the 3D hydrogel compared to 2D tissue culture plates. After 15 days of culture, 3D culture environment significantly improved the expression of Cardiac Troponin T (cTnT), Myosin heavy Chain 6 (Myh6) and Cardiac Troponin I (cTnI) in both miRcombo and negmiR transfected AHCFs compared to 2D cultures. However, cTnT and Myh6 expression in miRcombo-transfected AHCFs embedded in the 3D hydrogel was significantly higher compared to negmiR controls cultured in 3D system, suggesting the positive effect of a 3D biomimetic culture micro-environment on direct reprogramming of cells.

Conclusions

This study demonstrated that miRcombo-mediated direct reprogramming of human cardiac fibroblasts can be improved in a 3D culture system compared to 2D culture plates and that miRcombo transfection can enhance the expression of cardiomyocyte-specific mature markers.

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