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Background

Myocardial infarction (MI) remains one of the leading causes of death worldwide. MI results in the loss of up to 1 billion of cardiomyocytes (CMs) and the formation of a fibrotic scar, mainly populated by cardiac fibroblasts, which impair normal cardiac function [1]. Since the only therapeutic strategy nowadays is heart transplantation, the necessity to find a new approach to restore cardiac function is of huge importance. Direct reprogramming of human cardiac fibroblasts into CMs may hold a great potential for this purpose. Recently, direct reprogramming of murine fibroblasts into cardiomyocytes (CMs) using the combination of four different microRNAs (miR-1, 133, 208 and 499), named “miRcombo”, has been demonstrated [2,3]. Moreover, the use of miR-1 and miR-133 together with cardiac transcription factors (Gata4, Hand2 and Tbx5), has been demonstrated to induce direct reprogramming of human adult fibroblasts to a cardiac phenotype [4].

Methods

In the present study, we evaluated miRcombo mediated reprogramming of human adult cardiac fibroblasts (AHCFs) into CMs. After transfection with miRcombo, cells were cultured on 2D tissue culture plates or embedded into 3D fibrin hydrogel beads. Results were evaluated by analyzing gene expression by digital droplet PCR (ddPCR) and protein expression by immunocytochemistry at 4, 7 and 15 days.

Results

After 7 days in 2D culture, ddPCR analysis showed significantly enhanced expression of cardiac transcription factors (TFs) such as Gata4, Mef2c, Tbx5 and Hand2 compared to controls. Immunocytochemical analysis showed increased expression of late cardiac markers α-sarcomeric actinin and cardiac Troponin T (cTnT) in

miRcombo-transfected AHCFs after 2 weeks of culture in 2D. However, ddPCR showed no significant differences of late cardiac markers such as Myosin heavy chain 6 (Mhy6) and cardiac Troponin I (cTnI) expression between the groups after 2 weeks in 2D culture, meaning that additional time is needed to observe the expression of late cardiac markers. When miRcombo-transfected AHCFs were cultured in a fibrin-based 3D hydrogel, the expression of cardiac transcription factors (such as Gata4, Tbx5 and Nkx2.5) was enhanced compared to 2D culture after 4 days. After 2 weeks, miRcombo-transfected AHCFs cultured in 3D hydrogels showed a strongly enhanced expression of cardiac genes such as Myh6 and cTnI compared to 2D cultures.

Conclusion

Results showed that proper 3D environment plays a key role in enhancing direct reprogramming of AHCFs into CMs, needing further investigation.

References

[1] Paoletti C et al. *Cells* **2018**, 7, 114

[2] Jayawardena TM et al. *Circ Res.* **2013**, *110*, 1465–1473

[3] Li Y et al. *Sci. Rep.* **2016**, *6*, 1–11

[4] Nam YJ et al. *PNAS* **2013**, 110, 5588-5593

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