Therapeutic transdifferentiation of fibroblasts to functional endothelial cells upon shock wave therapy

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1. Introduction

Reprogramming of cardiac fibroblasts towards functional endothelial cells is a promising strategy for the vascular regeneration of ischemic myocardium. Recent studies reveal that stimulation of inflammatory signaling is required for effective chromatin remodelling and nuclear reprogramming. Mechanical conditioning of myocardium via shock wave therapy (SWT) has been shown to activate TLR3. We hypothesized that the activation of TLR3 via SWT might facilitate reprogramming of fibroblasts towards endothelial cells.

2. Material & Method

Human cardiac fibroblasts were treated with SWT or TLR3 agonist poly(I:C) in the presence of a specific induction medium known to promote endothelial lineage and analyzed for the expression of endothelial- specific markers. Induced endothelial cells (iECs) were subjected to functional endothelial cell assays including NO production and tube formation. iECs were suspended in matrigel and injected subcutaneously . A lineage tracing experiment was performed in a transgenic mouse model of Fsp1- Cre/LacZ mice after coronary occlusion and SWT. Myocardial scarring was evaluated histologically, whereas left ventricular (LV) function was assessed via transthoracic echocardiography. Chromatin remodeling and epigenetic plasticity were evaluated via Western Blot and ATAC sequencing.

3. Results

SWT activated TLR3 signaling and triggered the expression of endothelial genes in a TLR3 dependent fashion. SWT resulted in higher numbers of iECs. iECs were capable of producing endothelial nitric oxide (NO) and of forming tube-like structures. In vivo, the subcutaneous injection of iECs resulted in higher numbers of vessels and improved perfusion in a Matrigel plug assay. In a lineage tracing experiment in Fsp1-Cre/LacZ mice, we found higher numbers of LacZ/CD31 positive cells after coronary occlusion and subsequent SWT indicating transdifferentiation in vivo. Myocardial scar size was reduced after SWT, whereas LV function was improved. Mechanistically, SWT enhanced epigenetic plasticity via the TLR3 – NFkB - IL-6 –STAT3 – PRDM14 axis. SWT and Poly(I:C) induced significant changes in chromatin organization, with chromatin being more accessible after both treatments in 1705 genomic regions.

4. Discussion

We provide evidence for the induction of transdifferentiation in ischemic myocardium via SWT. Therapeutic transdifferentiation may contribute to the beneficial effects of SWT in the clinic.