Mechanical Stretch Promotes Pluripotent Stem Cell Cardiac Differentiation via miR-1-Dependent Signaling

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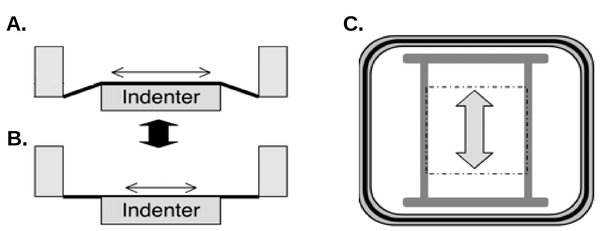
Background

Heart failure is attributable to the programmed cell death of cardiac muscle cells and the inflammatory cell response that prevent adult mammals from appropriately regenerating vital cardiomyocytes. This research intends to establish conditions that simulate the native cellular environment of a beating heart in vitro that is optimal for cardio-differentiation to compensate for lost cells. There are three key aspects to this research: pluripotent stem cell (PSC) differentiation, mechanical stretch application, and microRNA-1 (miR-1) genetic signaling. PSCs are the ideal cell source because of their capacity to differentiate into cardiomyocytes under the appropriate physical-chemical conditions. Mechanical stretch is a physical force that has been demonstrated to mediate the expression and activation of particular genes and proteins that regulate cardiac development. MicroRNA are short noncoding genetic sequences that suppress target proteins. MiR-1 is associated with smooth muscle protein suppression, thus allowing for cardiac protein expression. We will subject PSCs to mechanical stretch during cardiac differentiation while quantifying miR-1 expression levels. The aim is to elucidate a detailed mechanism of how mechanical stretch promotes PSC-cardiac differentiation through miR-1- dependent signaling.

Methods

Differentiation protocol: We followed the instructions in the Sigma Aldrich cardiomyocyte differentiation protocol.

Stretching experiments: Human PSCs (H1 cells) from 4 differentiation stages were seeded on fibronectin-coated silicon sheets (0.01mm thickness) at a concentration of 40,000 cells/chamber (2×2cm^{2}surface area). Cells were incubated for 24h before applying uni-axial cyclic mechanical stretch (10% deformation, 1.25 Hz) for 24h. Controls were non-stretched (static) cells cultured on the stretch chambers.



Schematic drawings of the stretch chamber. (A, B) A side view of the chamber: the membrane is cyclically pushed down against the indenter to create stretch and returned to original position. (C) Top view of the stretch chamber.

Specific Aims

1) To elucidate the mechanisms by which mechanical stretch promotes PSC-cardiac differentiation through miR-1-dependent signaling.

2) To identify a novel target protein of miR-1 with regards to stretch sensing

Results

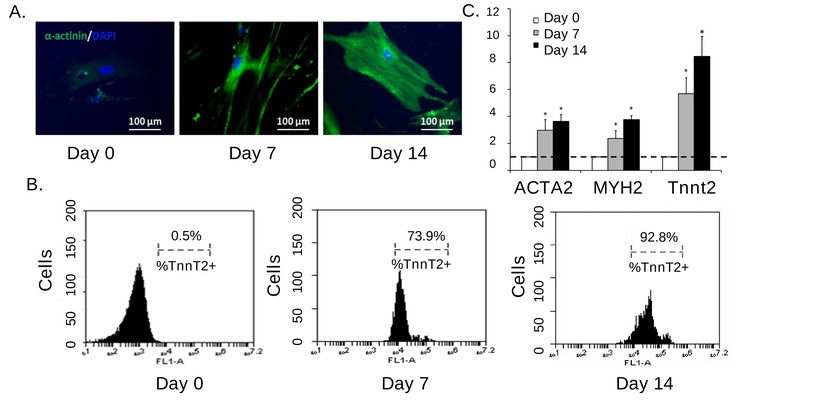


Figure 1. Biochemical induction of PSC-cardiomyocyte differentiation. The expressions of cardiac-specific markers, (A) α-actinin, (B, C) ACTA2, MYH2 and TnnT2 increased in PSCs upon differentiation induction throughout 14 days.

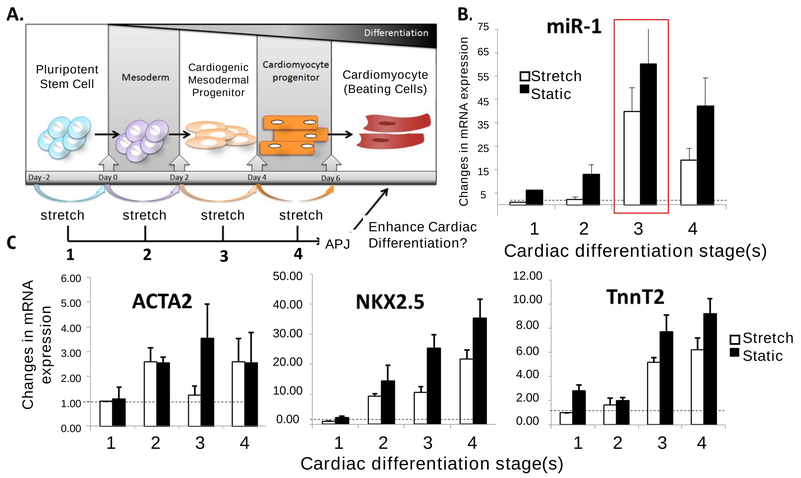


Figure 2. Effect of cyclic stretch on PSC-cardiomyocyte differentiation. Experimental design of stretch at 4 different stages during PSC-cardiac differentiation. Mechanical stretch increased (B) miR-1 expression and (C) ACTA2, NKX2-5 and TnnT2 in PSCs at 4 differentiation stages.

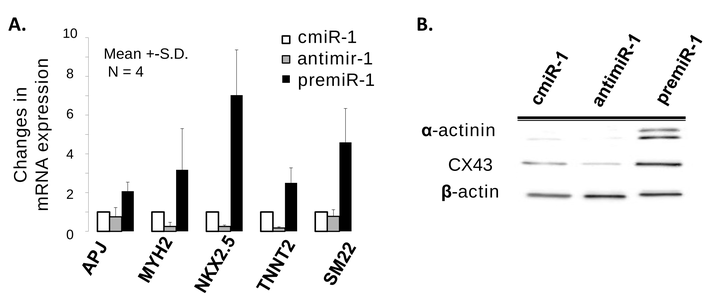
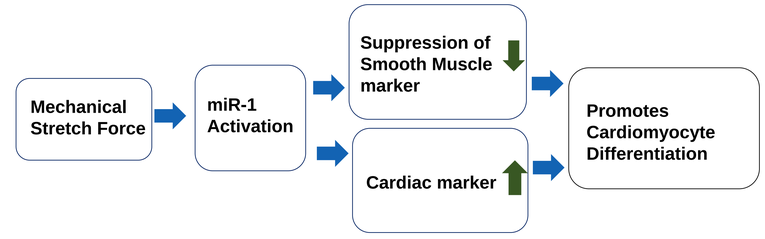


Figure 3. Effect of miR-1 on PSC-cardiomyocyte differentiation. Pre-miR-1 treatment increased the (A) gene and (B) protein expression levels of cardiac markers, and anti-miR-1 treatment exhibited opposite effects.

Conclusion



We demonstrate that mechanical stretch promotes miR-1 expression to increase cardiomyocyte marker genes. We hypothesize that mechanical stretch promotes PSC-cardiac differentiation through miR-1-dependent mechanotransduction by directly targeting the smooth muscle cell markers or anti-cardiac differentiation markers.

Future Work

To understand the detailed mechanisms by which miR-1 mediates cardiac function and development, we used an in silico approach to predict the miR-1 targets and their potential binding sites in PSCs.

MiR-1 candidate targets.

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(1) BDNF mRNA- 5'...UAAAAAGUCUGCAUUACAUUCCU...
                        |||||||
    miR-1       3'     UAUGUAUGAAGAAAUGUAAGGU


(2) PAX7 mRNA- 5'...CCCUCAGUCAACAAGACAUUCCU...
                         |||||||
    miR-1       3'      UAUGUAUGAAGAAAUGUAAGG


(3) NFAT5mRNA- 5'  ...CUGUUCAUCUGUGGCCAUUCCAU...
                         ||||||
    miR-1       3'   UAUGUAUGAAGAAAUGUAAGGU
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Figure 4. Three potential targets were identified:
(1) brain-derived neurotrophic factor (BDNF), a factor that involves in the cardiac aging and inflammation signaling transduction, (2) paired-box transcription factor 7 (Pax7), a key factor to control primary myoblasts differentiation, (3) nuclear factor of activated T-cells 5 (NFAT5), a transcription factor that regulates the expression of genes involved in the osmotic stress and associate with pro-inflammatory cytokine production.

We will knockdown the miR-1 in PSCs to investigate the role of miR-1 in regulating these identified targets and their functional consequences during cardiomyocyte differentiation.

We will construct luciferase reporters with the full-length 3'UTR of these identified potential miR-binding sites. The 3'UTR-luciferase will be co-transfected into PSCs together with either the anti-miR-1 or pre-miR-1 to elucidate the direct interactions between miR-1 and 3'UTR binding sequences.