Actomyosin−mediated cellular tension promotes Yap nuclear translocation and myocardial proliferation through a5 integrin signaling

2022 Lifespan Research Day Abstract

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Abstract

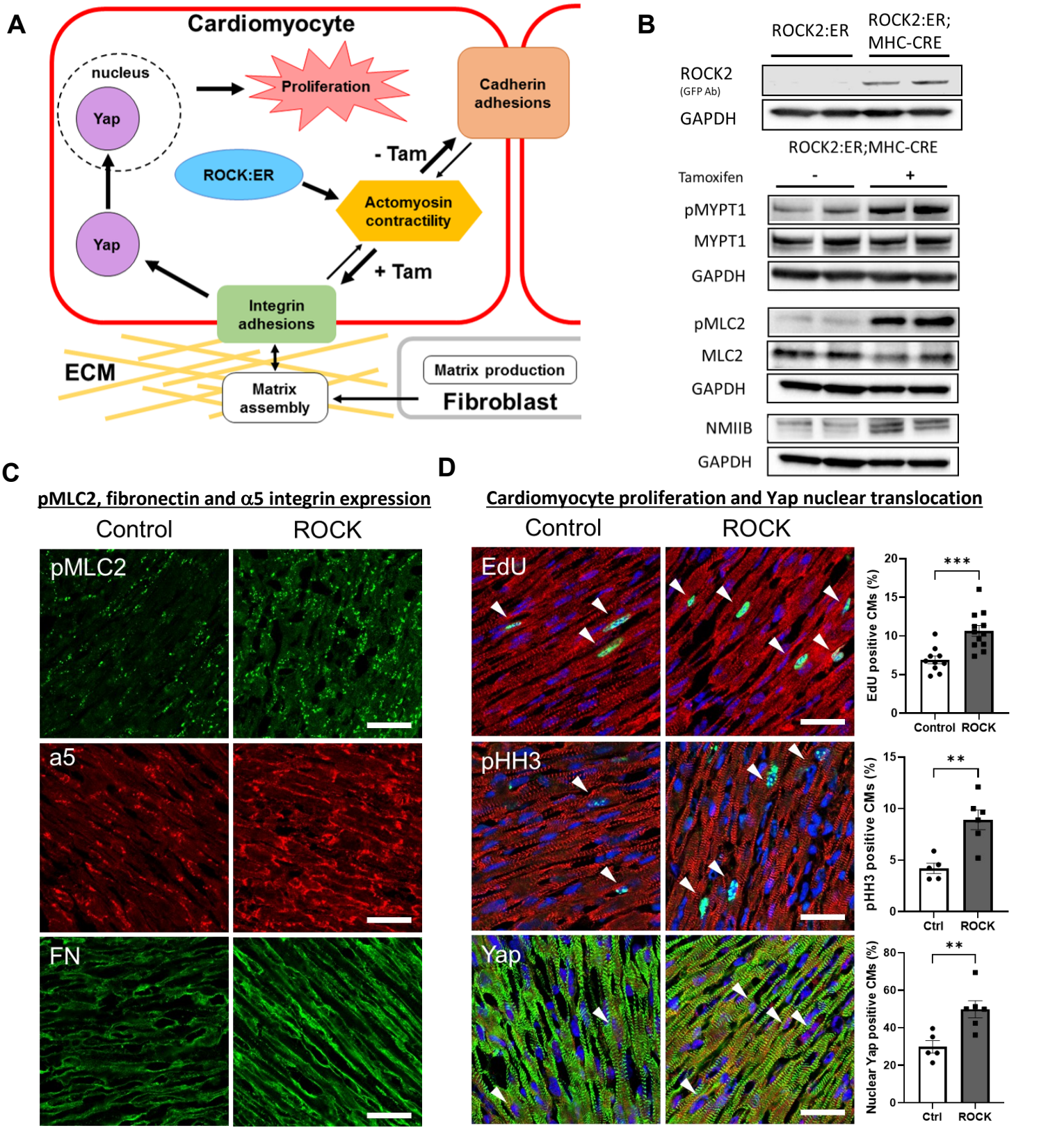
Background & Aim: When a heart attack happens, blood flow to the heart is blocked causing billions of heart muscle cells to die. Unfortunately, cardiomyocytes lose their ability to regenerate shortly after birth. Thus, the adult human heart cannot replace the lost cardiomyocytes following a heart attack. Thus, identifying novel targets that stimulate cardiomyocyte proliferation is urgently needed. Rho−associated protein kinase 2 (ROCK2) is a downstream effector of Rho pathway which regulates cytoskeletal tension. My project aims to determine whether modulating cytoskeletal tension will stimulate the mechanotransduction pathway that controls cardiomyocyte proliferation.

Methods: Using a novel mouse model, we induced ROCK2 activity in the neonatal heart during the period when cardiomyocyte proliferation ceases. To monitor cytoskeleton and junction remodeling, we used markers of actomyosin contractility and cell adhesion. To assess proliferation, we used markers of cell cycle activity including EdU incorporation, pHH3, and transcriptional co−activator Yap.

Results: We found that activation of ROCK2 led to increased cytoskeletal tension, and enhanced cell−extracellular matrix (ECM) interactions via upregulation of a5 integrin and fibronectin. ROCK2 activated cardiomyocytes exhibited increased nuclear Yap and increased proliferation in the neonatal heart. Reduction of myocardial a5 integrin rescues the myocardial proliferation phenotype in ROCK2:ER hearts

Conclusion: We demonstrated that cardiomyocytes respond to increase intracellular tension by altering their intercellular contacts in favor of cell−matrix interactions leading to Yap nuclear translocation, thus promotes cardiomyocytes proliferation.

Clinical Implications: The study led to an understanding of how cardiomyocytes coordinate signals from the actin cytoskeleton, adhesion systems into a proliferative response and may suggest new therapeutic strategies to stimulate cardiac regeneration after a heart attack.



(A) Working model for cytoskeletal regulation of cardiomyocyte (CM) proliferation.
(B) Western blot analysis of ROCK2, phosphorylation of substrates MYPT1 and MLC2, and NMIIB expression in P5 ROCK2:ER and control hearts after tamoxifen treatment.
(C) Representative immunofluorescent images of P7 ROCK2 heart sections stained pMLC2, α5 integrin, and fibronectin.
(D) *Left:* Representative immunofluorescent images of P7 heart sections from control and ROCK mice were co-stained with EdU (green)/α-actinin (red), pHH3 (green)/α-actinin (red), and Yap (red)/α-actinin(green). *Right:* Quantification of EdU positive CMs percentage at P7 (n=10-12), pHH3-positive CMs percentage at P7 (n=5-6), nucleus-Yap positive CMs percentage at P7 (n=5-6). Arrow heads indicate EdU positive CMs, pHH3 positive CMs, and nucleus-Yap positive CMs in ROCK:ER mice. ROCK:ER pups lacking the Cre transgene serve as controls. n: animal number. A minimum of 10 fields were analyzed per animal. **P<0.01, ***P<0.001 by Student's t-test. Scale bar: 25μm. Error bars represent S.E.M.