Reduced matrix rigidity promotes cardiomyocyte proliferation, dedifferentiation, and clonal expansion

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Abstract

Cardiomyocytes (CMs) in vertebrates lack significant regenerative potential; these cells withdraw from cell cycle shortly after birth. Recent publications indicate that CMs in zebrafish and neonate mammals can regenerate heart injury via proliferation of existing CMs. Mechanisms that increase the limited proliferative potential of CMs are intensively studied as cardiac regenerative strategies. Here, we explore the possibility that the mechanical properties of the CMs' microenvironment, specifically, matrix rigidity, affects CM proliferation and differentiation. We show that neonate CMs can be induced to proliferate by dedifferentiation and cell-cycle re-entry by manipulating matrix stiffness. We demonstrate, in neonatal rat and mouse models, an increase in the proliferative capacity of CMs cultured on soft substrates of 5kPa, and 20kPa, relatively to rigid 2MPa. Furthermore, soft matrices induced CM cell rounding and sarcomeric disorganization, characteristics of immature, dedifferentiated CMs. Live-cell high-resolution monitoring of transgenic αMHC-Cre X dTomato-lox - derived CMs revealed two distinct proliferative phenotypes that have differential CM cell fate outcomes; kariokinesis followed by cytokinesis, resulting in formation of new CMs, and karyokinesis without cytokinesis, leading to CM binucleation. Interestingly, cytokinesis of mononucleated, and to a lesser degree binucleated CMs, was induced by the 20kPa matrix. By correlating live-imaging with immunofluorescent microscopy and genetic lineage marking, we identified MHC $^{\text{lineage-positive}}$ CMs that completed cytokinesis and lost their sarcomeric protein expression. Importantly, the 20kPa substrate increased the frequency of MHC $^{\text{lineage-positive}}$ / cTnT $^{\text{negative}}$ CMs demonstrating that soft matrices promote dedifferentiation and proliferation of mature CMs. Moreover, using a clonal expansion assay on CMs derived from the *Confetti* reporter mice we demonstrate that the 20kPa matrix potentiated clonal expansion of the dedifferentiated CMs. Taken together, we propose that soft matrices provide a permissive microenvironment that enables CM dedifferentiation, proliferation, and expansion. This information can be exploited to derive new regenerative approaches to cardiovascular disease.