MECHANOSENSING PROTEINS YAP/TAZ DYNAMICALLY CONTROL CARDIAC CELL MATURATION AND FUNCTION BY SENSING ECM NANOTOPOGRAPHY

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Abstract

The composition and arrangement of extracellular matrix (ECM) components in adult cardiac tissue plays a key role in the organization and alignment of synchronously and functionally beating cardiomyocytes. Following Heart failure (HF) - the end stage of cardiac pathologies in which cardiac muscle is not able to pump adequate blood to the organism - critical changes in mechanics and nanotopography occur along with the composition of cardiac ECM. Such modifications in ECM compliance and nanotopography - collectively defined as remodelling - are deemed to hinder local cell viability and function. At the moment, reliable in vitro models of the dynamic modifications occurring in vivo during cardiac remodelling process are missing. The Mechanosensing pathway Hippo acts through its effectors YAP/TAZ and is sensitive to changes in ECM mechanical properties. In the present study we demonstrate that, while being responsive to substrate compliance in a pathophysiological range (0.3-40 kPa), the nuclear localization and transcriptional activity of YAP/TAZ can be tuned by dynamical modifications of surface mechanics and nanotopography, as demonstrated by ad hoc developed thermo-responsive polymers displaying shape-memory properties.

Keywords: Cardiac progenitor cells, mechanobiology, thermo-responsive polymers, Hippo pathway

1. INTRODUCTION

The nanostructure and chemistry of ECM is of utmost importance in the organization and alignment of synchronously and functionally beating cardiomyocytes in the adult heart. Modifications in ECM compliance and nanotopography - collectively defined as remodelling - are deemed to hinder local cell viability and function. Following myocardial infarction, the formation of a fibrotic scar occurs, and thus the surrounding tissue loses its compliance and contractility (Happe and Engler 2016). Changes in ECM mechanics and nanotopography are perceived by tissue-resident cells through a complex system of interconnected molecules collectively referred to as mechanosensing apparatus. This apparatus is entitled to transform and transduce biophysical cues coming from the surrounding ECM into a biological response, elicited at the gene expression level.

The Hippo signaling pathway effectors YAP/TAZ have been shown to be sensitive to changes in ECM mechanical properties while influencing stem cell shape, homeostasis and differentiation (Dupont et al 2011). This axis works by controlling the shuttling of the proteins YAP/TAZ from the cytoplasm to the nucleus, where they act as transcriptional co-activators. Hippo pathway has been shown to play a pivotal role during heart development by controlling cardiogenesis by cardiac cell hyperplasia, with its deregulation being crucial to the onset and progression of cardiac hypertrophy. YAP and TAZ paralog proteins are not expressed in mature cardiomyocytes *in vivo*, while being re-expressed right after myocardial infarction in cardiomyocytes and cardiac fibroblasts at the infarction border zone. Conversely, *ex vivo* adherent cells of any derivation (epithelial cells, fibroblasts, endothelial cells, cardiomyocytes) display a marked nuclear staining for YAP and TAZ when

grown on stiff surfaces like glass or polystyrene. In this work, we aimed at investigating the possibility that the nuclear shuttling and transcriptional activity of mechanosensing proteins YAP/TAZ can be controlled by substrate compliance in a pathophysiological range (kPa) in cardiac progenitor cells. Moreover, we studied whether YAP/TAZ nuclear shuttling can be controlled by dynamic changes in substrate elasticity and nanotopography. In doing so, we unfold an unprecedented strategy to dynamically control YAP/TAZ subcellular localization of YAP/TAZ and show that the proteins are crucial to determine cardiac progenitor cell fate decision.

2. MAIN TEXT

2.1. Methods

Adult human cardiac progenitor cells were obtained from human cardiac biopsies based on the expression of Stem Cell Antigen 1 (Sca-1). Polyacrylamide (PA) gels with stiffness values of 0.5, 10 and 40 kPa were prepared as described (Engler et al, 2004) with minor modifications. Glass round coverslips (Matsunami glass, Japan) were amino-silanized using Aminopropyltriethoxysilane (APES), whereas squared glass slides were fluoro-silanized by the use of Fluorosilane (Trichloro (1H, 2H, 2H-perfluorooctyl)silane) (Sigma Aldrich). Afterwards, PA gels were prepared by the addition of the adequate proportion of 40% (w/v) acrylamide stock solution (Sigma, USA) to 2% (w/v) bis-acrylamide (N,N'-Methylenebisacrylamide) (Tokyo Chemical Industry, Japan) stock solution and PBS, towards achieving the desired stiffness (Young modulus, E), according to the reported protocol. Polymerizing catalysts were added (10% (wv) ammonium persulfate (APS) at 1% of final volume and Tetramethylethylenediamine (TEMED) at 0.1% final volume). The mixture was poured between the fluorosilanized glass slide and aminosilanized coverslip for 30 min, after which the PA gel was attached to the aminosilanized coverslip. Finally, PA gels were functionalized with collagen (100 µg/mL) by the attachment to 0.2 mg/mL sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino)-hexanoate (sulfo-SANPAH; Pierce Biotechnology) covalently bound to the gel surface by photo-activation.

Thermo-responsive polymers displaying shape-memory properties were prepared by cross-linking tetra-branched Poly-ε-caprolactone (PCL) with acrylate end-groups in the presence of linear PCL telechelic diacrylates, according to a previously reported protocol (Mosqueira et al, 2014) To prepare shape-memory PCL substrates with permanent surface nanopattern, the PCL macromonomer solution was cured between a nanopatterned glass mold and a flat glass slide with a 0.2 mm thick Teflon spacer for 180 min at 80 °C. Nanopatterned molding of glass substrates was performed by using an electron beam (EB) lithography system (ELS-7500EX, Elionix, Hachioji, Japan). To program temporary surface patterns, the films were compressed in a thermo chamber. A compressive stress of 0.1 MPa was applied to the samples at 37 °C and maintained for 5 min. The embossing stress was then released at 4 °C after 10 min of cooling. Samples had a temporary surface pattern that could be triggered to transition to the permanent surface pattern by heating. YAP/TAZ expression and subcellular localization in the cells were followed by immunofluorescence. Nuclei were counterstained with DAPI.

2.2. Results and Discussion

Similarly to what shown in other cells types like mesenchymal stem cells and fibroblasts, in adult cardiac progenitor cells (CPCs), the intracellular localization of YAP/TAZ is sensitive to substrate compliance. In fact, while being restricted to the cytoplasm on soft poly-acrylamide surfaces (0.3-0.5 kPa), the proteins are strongly stained in cell nuclei on stiff surfaces (10, 40 kPa and MPa range). This result was confirmed independently of surface composition and with different cell types, thus pointing at YAP/TAZ as primary sensors of ECM stiffness. Following this experiment, two classes of thermo-responsive polymers were designed: the first preparation was able to shift its stiffness when the temperature was modified from 32 to 37°C (Young

Modulus$_{32}$= 20 MPa; Young Modulus$_{37}$= 0.91 MPa). The second polymer was designed as to modify its nanotopography when the same shift in temperature was imposed.

When CPCs were seeded onto thermo-responsive PCL surfaces with controlled stiffness in the MPa range and the temperature modified, a clear, although transient relocation of the proteins YAP/TAZ to the cytoplasm was noticed within 90 minutes. The nuclear expression of the proteins was promptly recovered within 180 minutes (**Figure 1**).

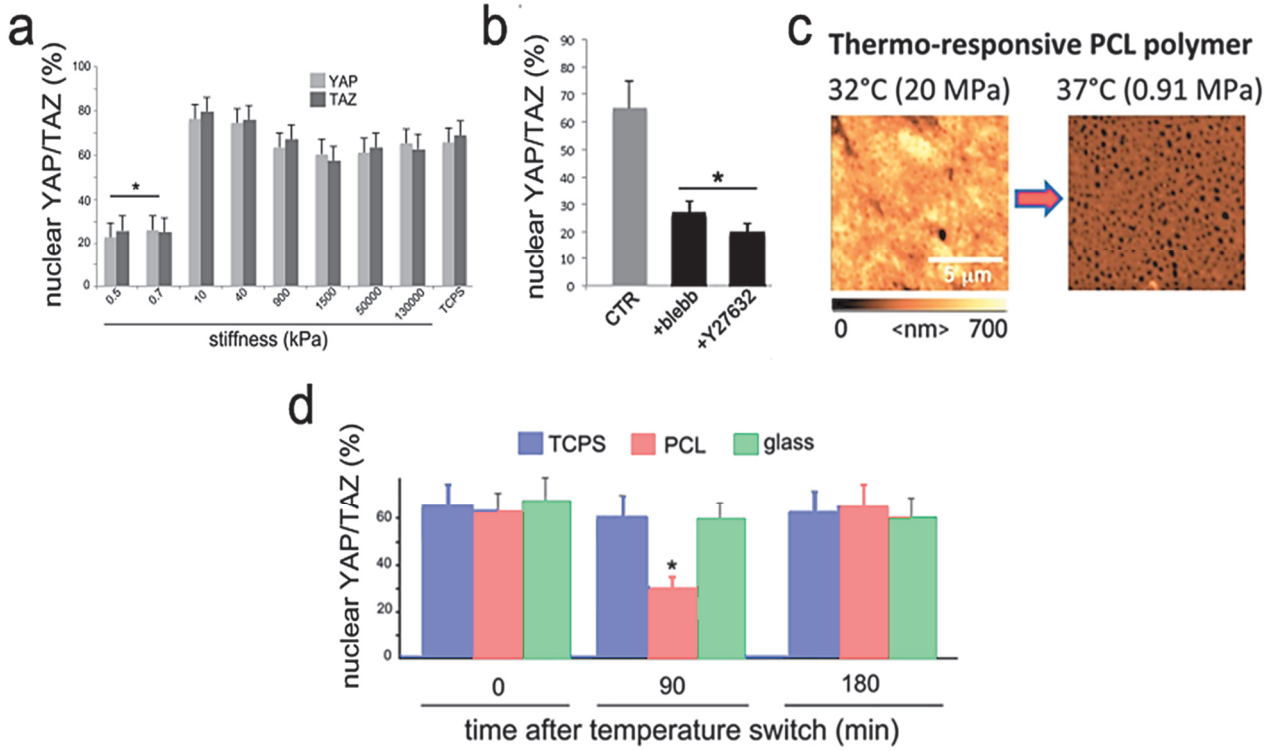

Figure 1 YAP/TAZ localization is dependent on substrate compliance (a) and the tension propagated through cell cytoskeleton (b). Thermo-responsive polymers were prepared as to display a dynamic switch in elasticity following a change in temperature (from 32 to 37°C, c). YAP/TAZ nuclear expression can be controlled by dynamic changes in substrate stiffness, as induced by thermal transition. As controls, the same temperature shift did not cause any shuttling of the proteins in cells grown onto glass or tissue culture polystyrene surfaces (TCPS, d)

The existence of a nanopattern in the second preparation of PCL surfaces was confirmed by AFM (SPM-9500J3, Shimadzu Co., Kyoto, Japan) in non-contact mode using a Si3N4 cantilever (spring constant; 42 N m−1), and with the sample temperature controlled. Additionally, by changing the temperature from 32 to 37 °C, we confirmed that the nanotopography of the surface could be dynamically modified within a limited time (90 minutes). These experiments helped us to clarify that these classes of polymers displayed a shape-memory effect (**Figure 2a**). To test the responsivenes of YAP/TAZ proteins to nanopattern modification, cardiac progenitor cells were seeded onto thermo-responsive polymers displaying shape-memory effect and a shift in surface nanotopography was imposed by switching the temperature from 32 to 37°C. YAP/TAZ proteins were stained with specific antibodies after 90 and 180 minutes. This experiment highlighted that YAP/TAZ proteins respond to dynamic modifications in substrate nanotopography by relocating to cell cytoplasm (90 min). After the nanotopography of the substrate is settled back at 37°C (180 min), the proteins shuttle back to the nucleus (**Figure 2**).

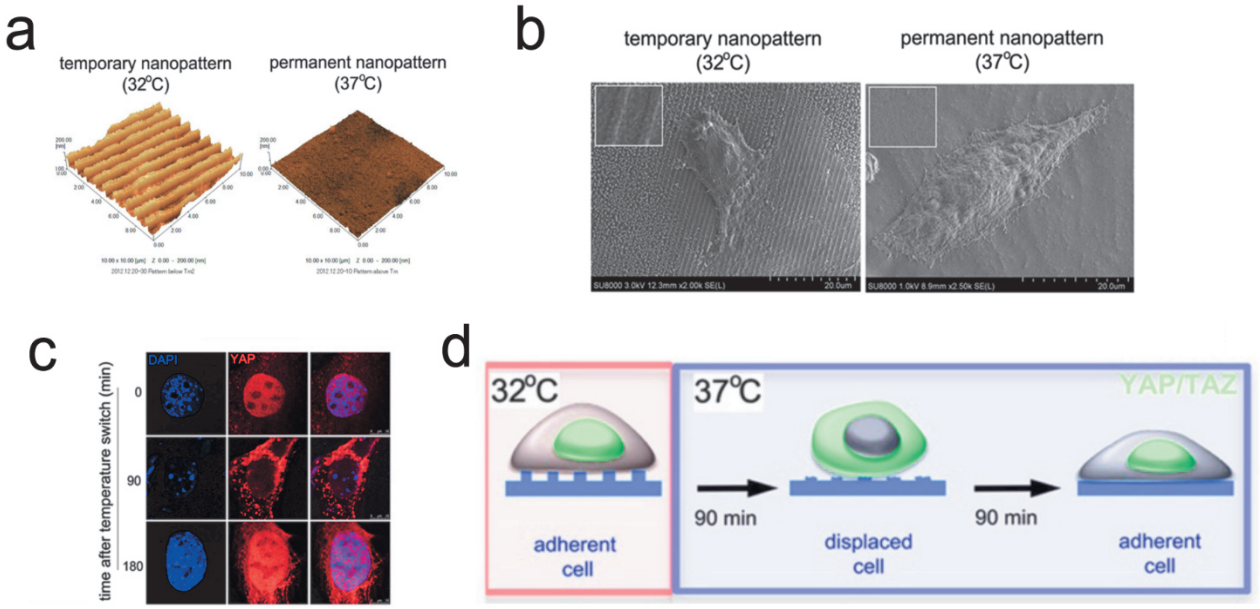

Figure 2 Thermo-responsive polymers displaying shape-memory effect can be induced to a transition by temperature switch, like shown in AFM analysis (a). The modification in substrate nanotopography causes a displacement in cell adhesion (b) and a temporary derangement in YAP/TAZ expression: the proteins exit the nucleus and are sequestered in the cytoplasm for a short time (90 minutes), until the stability of the surface is recovered. After 180 minutes the proteins shuttle back to the nucleus (c).
The process is depicted in (d)

Following experiments clarified that YAP/TAZ are also involved in CPC fate decision, by controlling the acquisition of the contractile phenotype. In fact, when the proteins were silenced in CPCs induced to differentiate towards the contractile phenotype, a clear reduction in the expression of key cardiac genes alpha cardiac actin, Nkx-2.5, myosing heavy chain was detected (**Figure 3**).

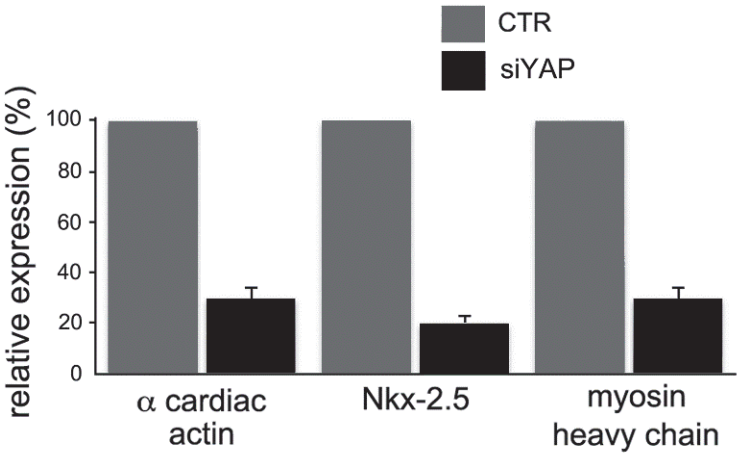

Figure 3 YAP-silenced cells display a reduced tendency to acquire cardiac mature phenotype when stimulated with cardiogenic factors. Real time PCR analysis of key cardiac genes like alpha (α) cardiac actin, Nkx-2.5 and myosin heavy chain were significantly reduced in cardiac progenitor cells as compared to their controls

3. CONCLUSION

In conclusion, in the present study we confirmed that adult tissue-resident cardiac progenitor cells possess an intrinsic and functional mechanosensing system based on the nuclear shuttling and transcriptional activation of YAP/TAZ proteins. These proteins are sensitive to substrate compliance, since they are relocated to the cytoplasm when cells are grown onto soft surfaces (0.3, 0.5 kPa) while being predominantly nuclear on stiff (10, 40 kPa) substrates. More importantly, we demonstrated for the first time that in cardiac progenitor cells YAP/TAZ protein localization can be dynamically controlled by modifying substrate stiffness and nanotopography through slight switches in temperature. This result was achieved by designing novel thermo-responsive polymers displaying shape-memory properties and represent a significant advancement to reproduce in vitro models of cardiac pathologies by mimicking the onset of ventricle remodeling. Moreover, given the role of YAP/TAZ in cardiac cell differentiation, such a result paves the way to novel approaches to control stem cell fate through active biomaterials.

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