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The response of human induced pluripotent stem cells to cyclic temperature changes explored by BIO-AFM

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Abstract

Human induced pluripotent stem cells (hiPSCs) are highly sensitive to extrinsic physical and biochemical signals from their extracellular microenvironments. In this study, we analyzed the effect of cyclic temperature changes on hiPSCs behaviors, especially by means of scanning force microscopy (BIO-AFM). The alternation in cellular mechanics, as well as the secretion and pattern of deposition of extracellular matrix (ECM) protein in hiPSCs were evaluated. The arrangement of the actin cytoskeleton changed with the variation of the temperature. The rearranged cytoskeleton architecture led to the subsequent changes in cell mechanics (Young's modulus of hiPSCs). With the exposure to the cyclic cold stimuli, an increase in the average surface roughness (Ra) and roughness mean square (RMS) was detected. This observation might be at least in part due to the upregulated secretion of Laminin α5 during repeated temporary cooling. The expression of pluripotent markers, NANOG and SOX2, was not impaired in hiPSCs, when exposed to the cyclic cold stimuli for 24 h. Our findings provide an insight into the effect of temperature on the hiPSC behaviors, which may contribute to a better understanding of the application of locally controlled therapeutic hypothermia.

Introduction

Human induced pluripotent stem cells (hiPSCs)-based models are promising tools for the investigation of stem cell fate and the development of patient-specific diseases models [1, 2]. In their extracellular environment, hiPSCs are exposed to multiple mechanical stimuli that regulate their fate [3–5]. As pluripotent cells, hiPSCs are able to adapt to those stresses and prevent themselves from damage [6, 7]. It showed that

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mechanosensitive ion channels were able to detect and channel the stresses [8], as the activated channels allowed the influx of cations and led to the cytoskeleton remodeling [9, 10]. The downstream signaling crosstalk, such as the Hippo/YAP pathway and Ras-activated signaling pathway, was then activated to counteract the stress [11–13].

Another important stress response of the cells is the thermal response [14, 15]. Cells have a variety of adaptive mechanisms to withstand temperature changes [16]. Heat stress is encountered in numerous medical conditions, such as inflammation [17], which led to the activation of heat shock proteins [18, 19]. Cold stress resulted in losing the cell membrane permeability, altering the enzyme activity, and modifying the cytoskeletons [20–23]. It should be noted that the temperature affects the supramolecular structures and conformations of extracellular matrix (ECM) proteins [24]. Temperature change (from 98 to 320 K) led to an expansion of total volume of protein (0.4% per 100 K) [25]. In the solution, the assembly of ECM protein was favored by increased temperature, as the attraction to the net force increases with temperature from 5 to 35 °C [26]. Consequently, cellular behaviors were altered by changes in ECM [27]. It is intrigued that the cold responses was not the same among different animal species, and the molecular basis in cold adaption is remained to be elucidated [23, 28]. Here, we

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examined the influence of cyclic temperature changes (ΔT) on the interaction between the cells and the ECM, including the cell shape and mechanical properties as well as the ECM topography.

Method

Detailed information regarding the BIH-001A cell line, cell culture, and fluorescence staining appear in Supplementary Method S1. The topography of decellularized extracellular matrix (dECM) and the mechanics of the hiPSCs were determined using an atomic force microscope (NanoWizard 4, JPK BioAFM, Germany). Decellularization was using the following protocol [29]. Briefly, samples were washed with 0.1% (w/v) sodium dodecyl sulfate (Sigma-Aldrich, Germany) for 1 h at room temperature. DNA contents were removed using 10 U/ml DNAse (Sigma-Aldrich, Germany). The topography of the dECM was measured using AC Mode in PBS at room temperature. The experiments involving living cells were conducted at 37 ± 0.1 °C controlled by a petri dish heater (JPK BioAFM, Germany). A tetrahedral tip (240-AC-NG, NanoAndMore GmbH, Germany) was used to measure the topography of the dECM and a colloidal tip (CP-qp-CONT-Au-B-5, NanoAndMore GmBH, Germany) to measure Young's modulus of a single living hiPSC (Fig. S1). Images and force curves were recorded by the JPK-SPM software (JPK BioAFM, Germany) and were processed using JPKSPM Data Processing software (JPK BioAFM, Germany). Detailed descriptions appear in the supplemental information.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). GraphPad Prism 8 (GraphPad Software, USA) was used for the statistical analysis using unpaired t-tests. $p < 0.05$ was considered statistically significant.

Results and discussion

Pluripotency of hiPSCs in response to cyclic temperature changes

The expression of NANOG and SOX2 was used to evaluate the pluripotency of hiPSCs (Fig. 1). A robust expression of the NANOG and SOX2 was observed, while an increase in SOX2 expression was detected in hiPSCs under the cyclic cold stress. As either higher or lower expression of SOX2 would lead to the loss of pluripotency [30, 31], our result implied that the cyclic cold stress might favor the ectodermal lineage commitment in hiPSCs through upregulation SOX2 [32–34]. In addition, the cell-intrinsic mechanics regulated the pluripotent state of the cells [35], this observation might also due to the changes in mechanical properties of hiPSCs.

Alterations in the rearrangement of cytoskeleton caused by cyclic temperature changes

The ability of a eukaryotic cell to maintain or change its shape depends on the cytoskeleton arrangement
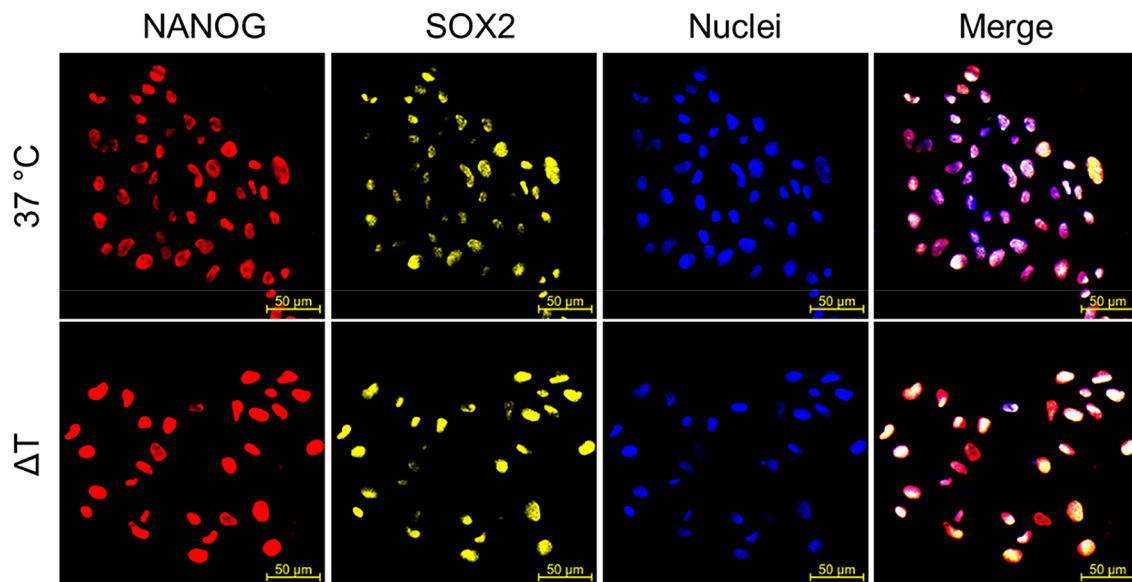
Fig. 1 Representative images of pluripotent markers expressed in hiPSCs with or without exposure to cyclic temperature changes for 24 h (scale bars are 50 µm)

[36]. Actin filament (F-actin) is one of the main types of cytoskeletal polymers, which regulates cell shape, mechanical properties, and intracellular signaling transduction [37]. Our result showed that, in both groups, thick F-actin fibers were displayed in parallel to the colony edge of hiPSCs, while a stronger fluorescence signal was detected in the hiPSC colony exposed to the cyclic temperature changes (Fig. 2a). At the single-cell level, more filopodia that formed by the long tight bundles of F-actin were observed in hiPSCs treated with the repeated temporary cooling. Without temperature change, the lamellipodia and cortical actin meshwork became apparent.

Alterations in the mechanics of hiPSCs treated with the cold stimuli

The cellular mechanical properties are dependent on the integrity and organization of the cytoskeleton [38]. We have demonstrated that the temperature change was a potent regulator of the expression and architecture of cytoskeleton component in the adult cells [39]. The value of Young's modulus in hiPSCs subjected to the cyclic temperature change was increased (Fig. 2b). The distribution of Young's modulus in hiPSCs at 37 °C was narrower than those exposed to the cyclic cold stimuli. The temperature-induced stiffening of hiPSCs was a result of the altered cytoskeleton arrangement [40, 41].
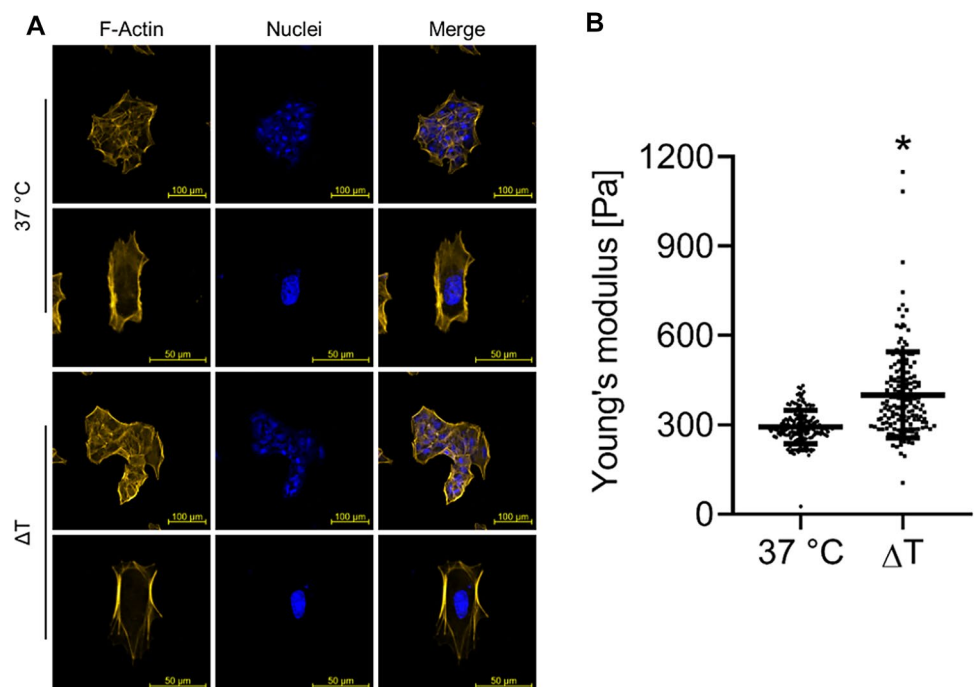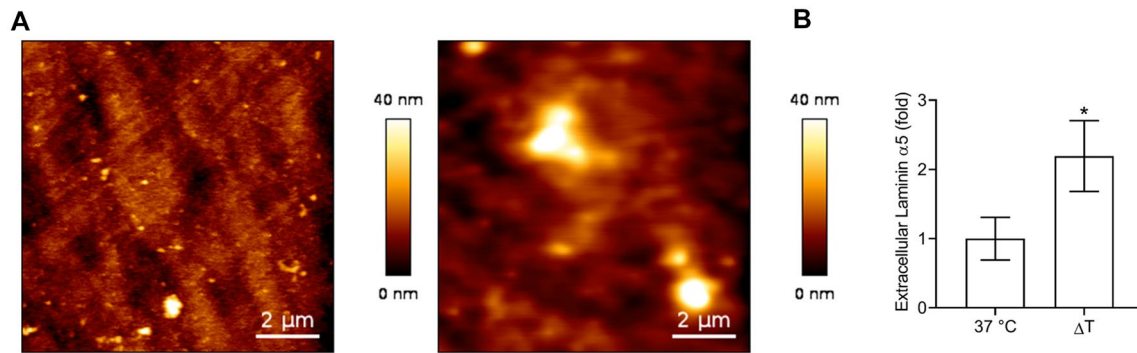
ECM remodeled by hiPSCs in response to cold stress

Through regulating the synthesis and secretion, assembly and reassembly, chemical modification, and degradation, ECM is constantly modified by the cells [42]. As the ECM become more and more stable along with its maturation, the remodeling of matured ECM would require proteases [43]. In addition, temperature change could affect the ECM assembly in the absence of cells [26]. The ECM topography was measured to reflect thermal influence on the capacity of hiPSCs to remodel their ECM using AFM (Fig. 3a). More surface incongruity was detected in the dECM derived from the cyclic cold-stimulated hiPSCs, as irregular larger peaks (the bright regions) with approximately 2 μm in width and 40 nm in height were observed.

The roughness average (Ra) and the root mean square (RMS) values were chosen to quantitatively characterize the surface roughness of the dECM (Table 1). In dECM derived from the hiPSCs exposed to the repeated temporary cooling, a significantly higher degree of roughness was exhibited. Previous studies demonstrated that cells could generate tractional forces through the cytoskeleton deformation to regulate the architecture of ECM. As the repeated temporary cooling altered the hiPSC cytoskeleton [44], it might thus cause changes in ECM arrangement.

Another possible reason for the altered ECM architecture is the production of ECM proteins by the cells. Laminin is one of the main components of ECM that involved in cell-ECM interactions and ECM remodeling [45, 46]. A substantial increase of Laminin α5 secretion was detected in hiPSCs

Fig. 2 Effects of cyclic temperature changes on hiPSC morphology and mechanical properties. **a** Representative images of F-actin organization in the hiPSC colony or in a single hiPSC cultured under different conditions for 24 h (scale bars for the image of hiPSC colony are 100 μm, for the single hiPSC are 50 μm). **b** Young's modulus of hiPSCs. Fifty-five single cells (biological replicates) were measured, each cell was measured three times (technical replicates), together 165 force curves were acquired for calculating Young's modulus of hiPSCs ($n = 165$; *$p < 0.0001$)

Fig. 3 ECM remodeled by hiPSCs in response to cyclic temperature changes. **a** Representative topography of dECM derived from hiPSCs with or without the cyclic temperature changes for 24 h. **b** Fold changes in the secretion of Laminin α5 in hiPSCs exposed to cyclic temperature changes for 24 h ($n = 3$; *$p < 0.05$)

Table 1 Mean ± standard deviation of Ra and RMS values for the dECM samples ($n = 10$, *$p < 0.0005$)

Culture condition	Ra (nm)	RMS (nm)
37 °C	31.56 ± 2.82	38.07 ± 3.58*
ΔT	39.52 ± 2.83	49.14 ± 4.23*

exposed to the cyclic cold stimuli (Fig. 3b). The difference in the secretion mode of Laminin α5 might be a result of the altered the arrangement of the cytoskeleton, as the actin cytoskeleton could play a regulatory role in the ECM protein secretion [47].

Conclusion

In this study, we assessed the responses of hiPSCs to the cyclic temperature changes, including the pluripotency, the cytoskeleton arrangement, and Young's modulus of the hiPSCs as well as their ability to remodel the ECM. Cyclic temperature changes did not decrease the expression of pluripotent proteins in hiPSCs, while it caused alterations in the arrangement of the actin skeleton, which further altered the ability of hiPSCs to remodel the ECM. The dECM in the group with cyclic temperature change presented increased surface roughness. These results provide insight into how hiPSCs adapt to the signals in the ambient environment, which might deepen the understanding of the cold-adaptive behaviors in stem cells and uncover the potential of therapeutic hypothermia.

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Declarations

Conflict of interest The authors declare no competing financial interest.

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