Stem Cell Research 17 (2016) 222–227

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Short report

ROCK inhibitor primes human induced pluripotent stem cells to selectively differentiate towards mesendodermal lineage via epithelial-mesenchymal transition-like modulation

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ARTICLE INFO

Article history:
Received 29 March 2016
Received in revised form 26 July 2016
Accepted 29 July 2016
Available online 4 August 2016

Keywords:
Pluripotent stem cells
Y-27632
Differentiation
Mesendoderm
Ectoderm

ABSTRACT

Robust control of human induced pluripotent stem cell (hIPSC) differentiation is essential to realize its patient-tailored therapeutic potential. Here, we demonstrate a novel application of Y-27632, a small molecule Rho-associated protein kinase (ROCK) inhibitor, to significantly influence the differentiation of hIPSCs in a lineage-specific manner. The application of Y-27632 to hIPSCs resulted in a decrease in actin bundling and disruption of colony formation in a concentration and time-dependent manner. Such changes in cell and colony morphology were associated with decreased expression of E-cadherin, a cell-cell junctional protein, proportional to the increased exposure to Y-27632. Interestingly, gene and protein expression of pluripotency markers such as NANOG and OCT4 were not downregulated by an exposure to Y-27632 up to 36 h. Simultaneously, epithelial-to-mesenchymal (EMT) transition markers were upregulated with an exposure to Y-27632. These EMT-like changes in the cells with longer exposure to Y-27632 resulted in a significant increase in the subsequent differentiation efficiency towards mesendodermal lineage. In contrast, an inhibitory effect was observed when cells were subjected to ectodermal differentiation after prolonged exposure to Y-27632. Collectively, these results present a novel method for priming hIPSCs to modulate their differentiation potential with a simple application of Y-27632.

1. Introduction

The derivation of human induced pluripotent stem cells (hIPSCs) has provided a technological foundation to produce a clinically-relevant, large quantity of cells, potentially offering a solution for cell source limitations in cell therapies and regenerative medicine (Romito and Cobellis, 2016; Zomer et al., 2015). Due to the difficulties in controlling behaviors of these cells in vivo, however, it is of great interest to develop in vitro methods/protocols to direct the differentiation of hIPSCs to specific phenotypes prior to implantation. In this regard, the physiochemical cues to direct lineage/phenotype-specific differentiation have been widely studied to identify the suitable physical and biochemical microenvironment to trigger specific signaling cascades mediating the subsequent differentiation process (Oldershaw et al., 2010; Shi et al., 2012; Zomer et al., 2015). Previous work in our lab demonstrated that varying the physical microenvironment, by means of scaffold mechanics, results in the development of distinct hIPSC colony morphologies with different cytoskeletal organization (Maldonado et al., 2015). The difference in cytoskeletal organization ultimately led to changes in IPSC behaviors, including self-renewal and lineage-specific differentiation potentials (Maldonado et al., 2015, 2016). Such a differential cytoskeletal organization has been associated with the RhoA activity in mesenchymal stem cells (McBeath et al., 2004). In this context, we hypothesized that modulation of the cell cytoskeleton via Rho-associated protein kinase (ROCK) inhibition would bias the differentiation potential of hIPSCs even in the absence of a physical microenvironment change.

Previous studies have focused on defining the role of Y-27632, a ROCK inhibitor, in preventing dissociation-induced apoptosis of human pluripotent stem cells (Emre et al., 2010; Watanabe et al., 2007). Although such efforts have provided an essential means to enhance hIPSC survival during cell expansion, we also observed that cell morphology was significantly affected by Y-27632. Based on this observation, we aimed to examine how pharmacological manipulation of cytoskeletal organization in hIPSCs modulates the self-renewal and differentiation potential of the cells. In this report, we show that a widely used small molecule Y-27632 primes hIPSCs to selectively differentiate towards mesendodermal lineage in an exposure duration-dependent manner. Specifically, the results suggest that the inhibition of ROCK initiates epithelial-mesenchymal transition (EMT)-like changes in hIPSCs to promote mesendodermal differentiation. Overall, we demonstrate a facile method to regulate cell and colony organization,

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http://dx.doi.org/10.1016/j.scr.2016.07.009

which in turn promotes the early stage differentiation of hIPSCs in a lineage-specific manner.

2. Materials and methods

2.1. Cell culture

Human induced pluripotent stem cells were derived as previously described (Maldonado et al., 2015). Cells were passaged using 0.25% Trypsin-EDTA (Life Technologies, Grand Island, NY) onto Geltrex®-coated tissue culture plastic or glass coverslips. To enhance initial cell survival, hIPSCs were seeded in the presence of ROCK inhibitor, Y-27632 (EMD Millipore, Billerica, MA) at 10 μM for 12 h, and the media was changed to regular maintenance media, mTeSR™1 (STEMCELL Technologies, Vancouver, Canada) for an additional 12 h to obtain a typical hIPSC cell/colony morphology. During the following pre-culture period, cells were exposed to Y-27632 with various concentrations and exposure durations, and subsequently subjected to gene and protein analyses. Alternatively, the cells after the pre-culture period were subjected to either a mesendodermal or an ectodermal differentiation protocol. For mesendodermal induction, temporally varied concentrations of Activin A (Peprotech, Rocky Hill, NJ), WNT3A (R&D systems, Minneapolis, MN), FGF2 (Invitrogen, Carlsbad, CA), and BMP4 (R&D systems) were used over the course of 60 h (Oldershaw et al., 2010). Specifically, day 1: WNT3A (25 ng/ml), Activin A (50 ng/ml), day 2: WNT3A (25 ng/ml), Activin A (25 ng/ml), FGF2 (20 ng/ml), day 3: WNT3A (25 ng/ml), Activin A (10 ng/ml), FGF2 (20 ng/ml), BMP4 (40 ng/ml). To induce ectodermal differentiation, the cells were subjected to a modified protocol using neurobasal media supplemented with N2 (Life Technologies), B27 (Life Technologies), 2 μM dorsomorphin (Sigma-Aldrich, St. Louis, MO), and 0.1 μM retinoic acid (Sigma-Aldrich) for 72 h (Shi et al., 2012). During the differentiation processes, the cells were optically observed by a BioStation CT (Nikon, Melville, NY) at the indicated time points. Analysis of lineage-specific gene expression was conducted after 60 h under mesendodermal differentiation condition or 72 h of ectodermal differentiation. To further confirm the differentiation tendencies of PSCs, a human embryonic stem cell (hESC) line WA09 (WiCell, Madison, WI) was also exposed to a similar culture regimen to analyze their differentiation tendencies.

2.2. Immunocytochemistry

To analyze hIPSC morphology and expression of lineage-specific markers, cells treated with various conditions were fixed with 4% paraformaldehyde for 30 min. After rinsing with phosphate buffered saline (PBS), the fixed cells were permeabilized using a 0.1% Triton-X solution. The cells were then blocked with a 1% BSA in PBS solution for 30 min followed by incubation with respective primary antibodies (mouse anti-NANOG (R&D systems), rabbit anti-E-cadherin (Abcam, San Francisco, CA), goat anti-BRACHYURY (R&D Systems), or mouse anti-PAX6 (DSHB, Iowa City, IA)) at 4 °C overnight. The samples were stained with their respective secondary antibodies (donkey anti-mouse 488, goat anti-mouse 594, goat anti-rabbit 488, or donkey anti-goat 488 (Invitrogen)). Actin and nuclei were counter-stained using rhodamine phalloidin (Life Technologies) and DAPI (4′,6-diamidino-2-phenylindole, Vector Laboratories, Burlingame, CA), respectively. Images were acquired using a Nikon Eclipse microscope and Image J software was used to quantitatively assess cell morphology and staining intensity.

2.3. Gene expression analysis

To quantify gene expression, an RNeasy Micro kit was used to extract total RNA from samples (Qiagen, Valencia, CA) and an iScript cDNA Synthesis Kit was used for cDNA synthesis (Bio-Rad, Hercules, CA) as described previously (Maldonado et al., 2015). The following custom primer sequences were used for rt-PCR gene expression analysis: GAPDH [5′-ATGGGGAAGGTGAAGGTCG-3′ (forward) and 5′-TAAAAG CAGCCCTGGTGACC-3′ (reverse)]; OCT4 [5′-TCCCAGGACATCAAAGCT CTG-3′ (forward) and 5′-CATCGGCCTGTGTATATCCCA-3′ (reverse)]; NANOG [5′-GCTTATTCAGGACAGCCCTGA-3′ (forward) and 5′-TTTGCG ACACTCTTCTCTGCA-3′ (reverse)]; FN1 [5′-CCCAATTCCTTGCTGGTA TCA-3′ (forward) and 5′-TATTCGGTTCCCGGTTCCA-3′ (reverse)]; SNAI2 [5′-AGACCCTGGTTGCTTCAAGGA-3′ (forward) and 5′-CCTCAG ATTTGACCTGTCTGCA-3′ (reverse)]; SNAI1 [5′-CTCAGATGTCAAGAAG TACCAGTGC-3′ (forward) and 5′-ACTCTTGGTGCTTGTGGAGCAG-3′ (reverse)]; LEF1 [5′-GAGCACTTTTCTCCAGGATCACA-3′ (forward) and 5′-ATCAGGAGCTGGAGGATGTCTG-3′ (reverse)]; T [5′-GGGTCCACAGCG CATGAT-3′ (forward) and 5′-TGATAAGCAGTCACCGCTATGAA-3′ (reverse)]; MIXL1 [5′-CTTTGGCTAGGCCGGAGATTA-3′ (forward) and 5′-GGCAGGCAGTTCACATCTACCT-3′ (reverse)]; SOX17 [5′-ACCGCACGGA ATTTGAACA-3′ (forward) and 5′-AGATTCACACCGGAGTCATGC-3′ (reverse)]; FOXA2 [5′-TCCATCAACAACCTCATGTCCT-3′ (forward) and 5′-CATCACCTGTTCGTAGGCCTTG-3′ (reverse)]; EOMES [5′-GATGGCGTGG AGGACTTGAAT-3′ (forward) and 5′-CGGTGTTTTGGTAGGCAGTCA-3′ (reverse)]; NODAL [5′-CTGGATCATCTACCCCAAGCA-3′ (forward) and 5′-ATGAAACTCCTCCCCAACAGG-3′ (reverse)]; PAX6 [5′-GAGTTCTTCG CAACCTGGCTA-3′ (forward) and 5′-CTGCCCGTTCAACATCCTTAG-3′ (reverse)]; SIP1 [5′-TCCACCTCAAAGCGCATTTC-3′ (forward) and 5′-GGTA TGGTCGTAGCCCAGGAAT-3′ (reverse)]; SOX1 [5′-AACGCCTTCATGGT GTGGT-3′ (forward) and 5′-TGATCTCCGAGTTGTGCATCTT-3′ (reverse)]; OTX2 [5′-AGAGCTAAGTGCCGCCAACAA-3′ (forward) and 5′-TTCCCGAGCTGGAGATGTCTT-3′ (reverse)]; NCAM1 [5′-GCGAGGTATT TGCCTATCCCA-3′ (forward) and 5′-CTGTAATTGGAGCTTGGCAGC-3′ (reverse)]. The data was analyzed using the comparative threshold cycle (C_T) method with *GAPDH* used as an endogenous control (Livak and Schmittgen, 2001).

2.4. Statistical analysis

All experiments were conducted at least in triplicate biological samples and data is represented as means ± standard error of means (SEM). SPSS (v.23.0) software was used to determine statistical significance using either one-way ANOVA with Tukey's post-hoc or one sample student *t*-test. Pearson's correlation coefficient was determined to reveal bivariate correlation between two factors. The difference was considered significant when a '*p*' value was <0.05.

3. Results and discussion

3.1. ROCK inhibitor modulates the colony and cellular morphologies of human induced pluripotent stem cells

A ROCK inhibitor, Y-27632, has been conventionally utilized on hIPSCs for its pro-survival effect after dissociation from their typical colony formation to single cells during culture (Emre et al., 2010; Watanabe et al., 2007). The application of Y-27632, even at lower concentrations, e.g., 10 μM Y-27632, accommodates cell attachment after dissociation and subsequent survival. However, such cellular behaviors are associated with the development of distinct cell morphologies, which is known to affect differentiation in adult stem cells (Nam et al., 2011). Thus, we first examined how different concentrations of Y-27632 affect the cell morphology and colony formation of hIPSCs. During culture in maintenance media (mTeSR™1) for 36 h with various concentrations of Y-27632, up to 50 μM, noticeable differences in cell detachment/apoptosis were not observed throughout the culture period regardless of the concentration (Fig. 1A–B). Significant changes in cell colony morphology, however, were observed with the application of Y-27632 (Fig. 1A–B). As the concentration of Y-27632 increased, the cells dispersed, forming loosely populated colonies. Albeit Y-27632 effectively disrupted hIPSC colony formation, the cells maintained expression of pluripotency marker NANOG for the examined duration. The

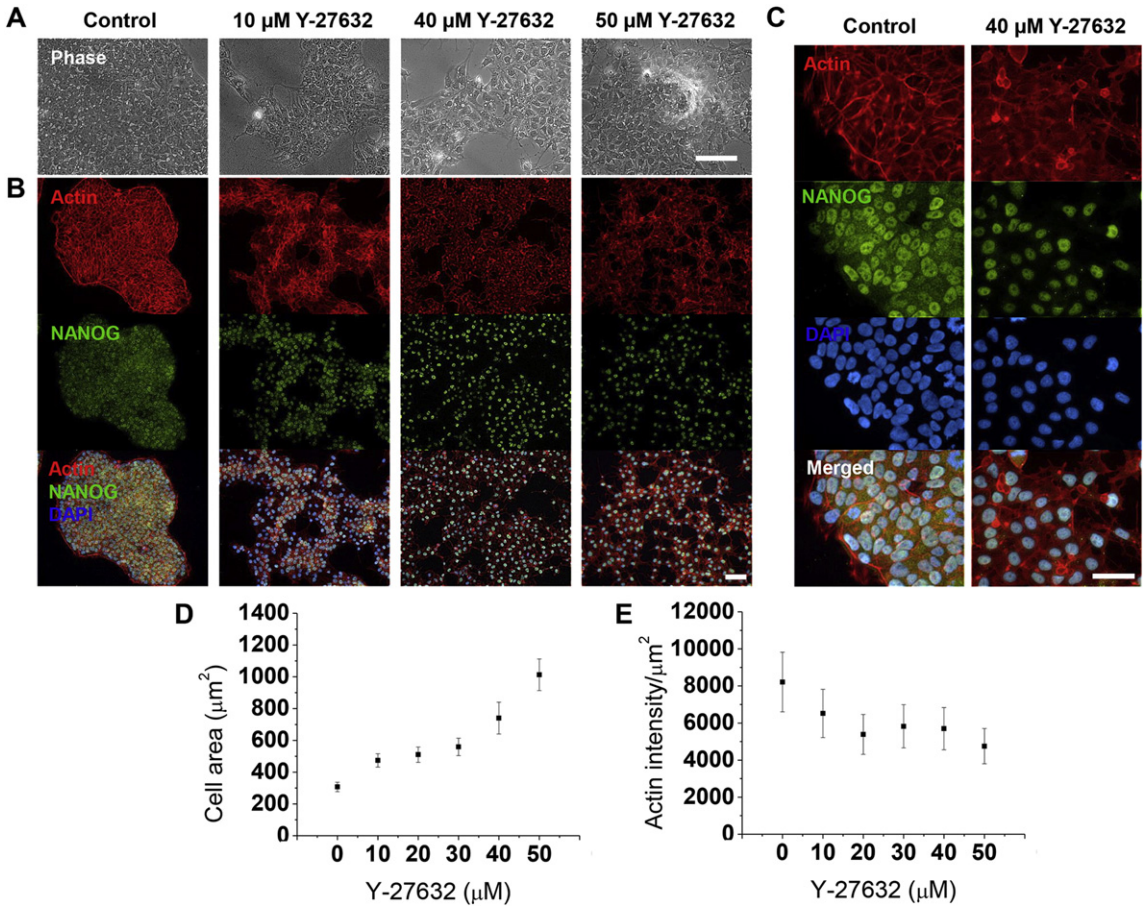

Fig. 1. Y-27632 modulates colony and cell morphologies of hIPSCs in a concentration-dependent manner. (A) Phase contrast and (B) immunofluorescent images showing that colony morphologies of hIPSCs were differentially regulated by the application of Y-27632 at various concentrations. Regardless of colony morphologies the cells maintained expression of pluripotency marker NANOG (red: actin, green: NANOG, blue: DAPI, scale bar: 100 μm). (C) High magnification fluorescent images of the actin cytoskeleton and NANOG expression revealed changes in cell spreading and cytoskeletal organization by Y-27632 (red: actin, green: NANOG, blue: DAPI, scale bar: 50 μm). (D) An increase in cell spreading was positively related to the concentration of Y-27632 ($n = 100$ cells, Pearson's correlation: 0.740, significance: 0.000). (E) Actin bundling in hIPSCs was inversely related to the concentration of Y-27632 ($n = 100$ cells, Pearson's correlation: −0.423, significance: 0.000). Cells were pre-cultured in mTeSR™1 media with various concentrations of Y-27632 for 36 h before each analysis.

changes in individual cell morphology were also observed by immunofluorescence imaging (Fig. 1C). Quantitative analysis of cell morphology based on the projected cytoplasmic area revealed greater cell spreading with the increased Y-27632 concentration (Pearson's correlation coefficient: 0.740, significance; 0.000) (Fig. 1D). The changes in cell shape were associated with cytoskeletal reorganization as evident from actin bundling changes, which was significantly decreased with the increased Y-27632 concentration (Pearson's correlation coefficient: −0.423, significance: 0.000) (Fig. 1E). Based on these observations, we utilized 40 μM Y-27632 for the subsequent experiments, which was the minimum concentration eliciting significant effects on the colony and cell morphologies of hIPSCs without altering their protein expression of pluripotency marker NANOG.

3.2. ROCK inhibitor affects hIPSC morphology and gene expression of EMT markers in an exposure duration-dependent manner

To explore the exposure duration-dependent changes in cell and colony morphologies, hIPSCs were cultured in the maintenance media supplemented with 40 μM Y-27632 for either 0, 2, 12, 24, or 36 h during the pre-culture period prior to the start of differentiation (Fig. 2A). Similar to our concentration study, cell and colony morphologies were significantly disrupted with the addition of Y-27632 as compared to the control (Fig. 2B). Before the supplementation with Y-27632, cells developed colonies with well-defined boundaries, but within 2 h after the addition of Y-27632 the cells at the periphery of the colonies developed extensions and exhibited an elongated, spread morphology (Fig. S1). A longer exposure to Y-27632 had more significant effects on cell morphology evident by a decrease in actin bundling (Fig. 2B). Such distinguishable differences in cell/colony morphology led us to examine differences in cell-cell junction protein E-cadherin (Fig. 2C). Increasing exposure to Y-27632 resulted in a remarkable disruption of E-cadherin expression at the cell junctions (Fig. 2C–D). For shorter exposure durations, the effects of Y-27632 were primarily localized to cells at the periphery of the hIPSC colonies. These findings were also confirmed with a human ESC line where a noticeable decrease in E-cadherin expression and actin bundling within the cytoplasm were observed (Fig. S3A). Because E-cadherin expression is one of the markers typically used to determine pluripotency in hIPSCs, we also examined the expression of pluripotency marker NANOG. No significant differences in NANOG expression were observed for the examined durations (Fig. 2C–D). Additionally, gene expression of *OCT4* and *NANOG*, pluripotent genes, were not significantly downregulated after 36 h of exposure to Y-27632 in both the hIPSC and hESC cell lines (Figs. 2E, S3B). A similar observation was noted in a study where the upregulation/maintenance of pluripotency markers was shown at the early stage of pluripotent stem cell differentiation to mesendodermal lineage (Oldershaw et al., 2010). Although we observed sustained expression of pluripotency markers, further analysis is required to determine if the cells truly maintain pluripotency. The cell-cell interaction changes in pluripotent stem cells are often associated with an EMT, which results in a loss of E-cadherin expression at the cell-cell junctions similar to our observation

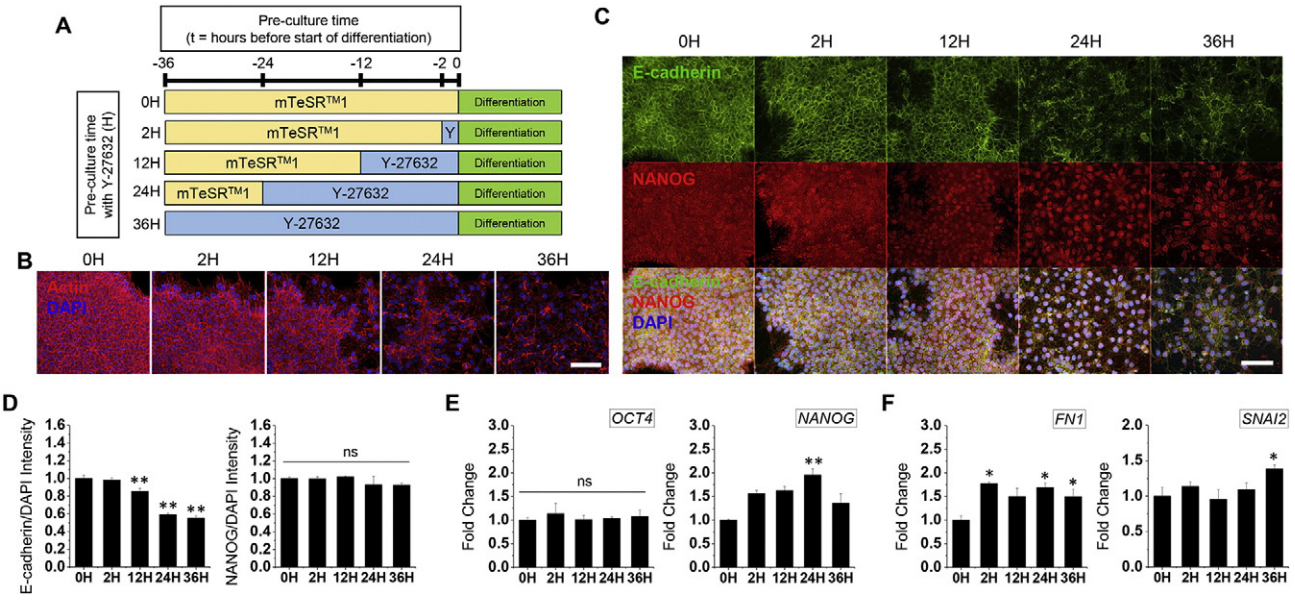

Fig. 2. Y-27632 induces cell spreading of hIPSCs, which increases expression of EMT markers, in an exposure duration-dependent manner. (A) A schematic of experimental design to examine the exposure duration-dependent effects of Y-27632 on hIPSCs. Cells were maintained in mTeSR™1 media supplemented with 40 μM Y-27632 for the indicated durations prior to being subjected to differentiation at $t = 0$. (B) Actin bundling decreased as the exposure duration to Y-27632 increased at $t = 0$ (red: actin, blue: DAPI, scale bar: 100 μm). (C) While longer exposure to Y-27632 decreased E-cadherin expression, hIPSCs maintained expression of pluripotency marker NANOG up to 36 h of the Y-27632 exposure at $t = 0$ (green: E-cadherin, red: NANOG, blue: DAPI, scale bar: 100 μm). (D) Quantification of E-cadherin and NANOG expression confirmed a decrease in E-cadherin intensity and insignificant change in NANOG expression by the increase in the exposure duration to Y-27632 ($n = 100$ cells, **: $p < 0.01$ as compared to 0H control, 'ns' = no significance). (E) Pluripotency markers (*OCT4* and *NANOG*) were not downregulated by the increased exposure to Y-27632. (F) Epithelial-to-mesenchymal transition (EMT) markers (*FN1* and *SNAI2*) were significantly upregulated with a 36 h Y-27632 exposure at $t = 0$ ($n = 6$, 3 biological samples with 2 technical duplicates, *: $p < 0.05$ and **: $p < 0.01$ as compared to 0H control, 'ns' = no significance).

in the current study (Thiery et al., 2009). To determine if the application of ROCK inhibitor induces such a transition, we next examined the gene expression of EMT markers in the cells stimulated by Y-27632 (Figs. 2F, S2). Increases in gene expression of EMT markers, *FN1*, *SNAI2*, *SNAI1*, and *LEF1*, were detected with increased exposure to Y-27632 (Figs. 2F, S2). These observations collectively suggest that the application of the ROCK inhibitor, in an exposure duration-dependent manner, primes the cells for EMT. In this regard, Richter et al. demonstrated the importance of EMT-related genes in mediating BMP4-stimulated mesendodermal differentiation of human embryonic stem cells; it was shown that differentiation was primarily localized in the cells at the edges of colonies, which displayed disrupted E-cadherin organization with enhanced cell migration (Richter et al., 2014). In comparison, our results demonstrate that Y-27632 effectively modulates the cell/colony morphology of hIPSCs similar to the migrating cells during EMT, which maintain a more spread morphology and exhibit a decrease in cell-cell junctional E-cadherin. Additionally, similar behaviors were observed in the hESC line (Fig. S3). Therefore, the morphological control of PSCs by Y-27632 may provide a means to prime the cells for subsequent differentiation.

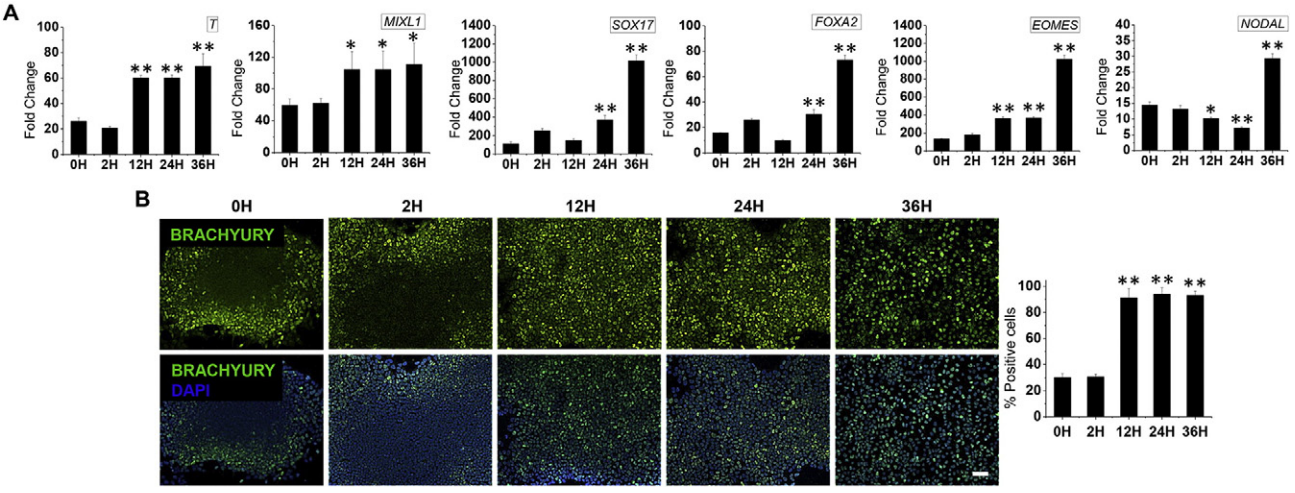

Fig. 3. Mesendodermal differentiation is enhanced by the application of Y-27632 during the pre-culture period in an exposure duration-dependent manner. Human IPSCs were pre-cultured for 36 h in mTeSR™1 media (supplemented with 40 μM Y-27632 for the designated durations such as 0, 2, 12, 24 and 36 h (Fig. 2A)) followed by subsequent WNT3A and Activin A-mediated mesendodermal differentiation for 60 h. (A) Gene expression of mesendodermal markers *T* and *MIXL1* was positively correlated to Y-27632 exposure duration. Endodermal markers (*SOX17* and *FOXA2*) and mesodermal markers (*EOMES* and *NODAL*) were also significantly affected by exposure to Y-27632 before the induction of differentiation ($n = 6$, 3 biological samples with 2 technical duplicates, *: $p < 0.05$ and **: $p < 0.01$ as compared to 0H control. Gene expression fold change values were normalized to that of hIPSCs not subjected to either Y-27632 or differentiation). (B) Immunofluorescent imaging and its quantitative analysis showed that BRACHYURY expression is positively correlated to Y-27632 exposure duration (green: BRACHYURY, blue: DAPI, scale bar: 100 μm; $n = 6000$ cells, **: $p < 0.01$ as compared to 0H control).

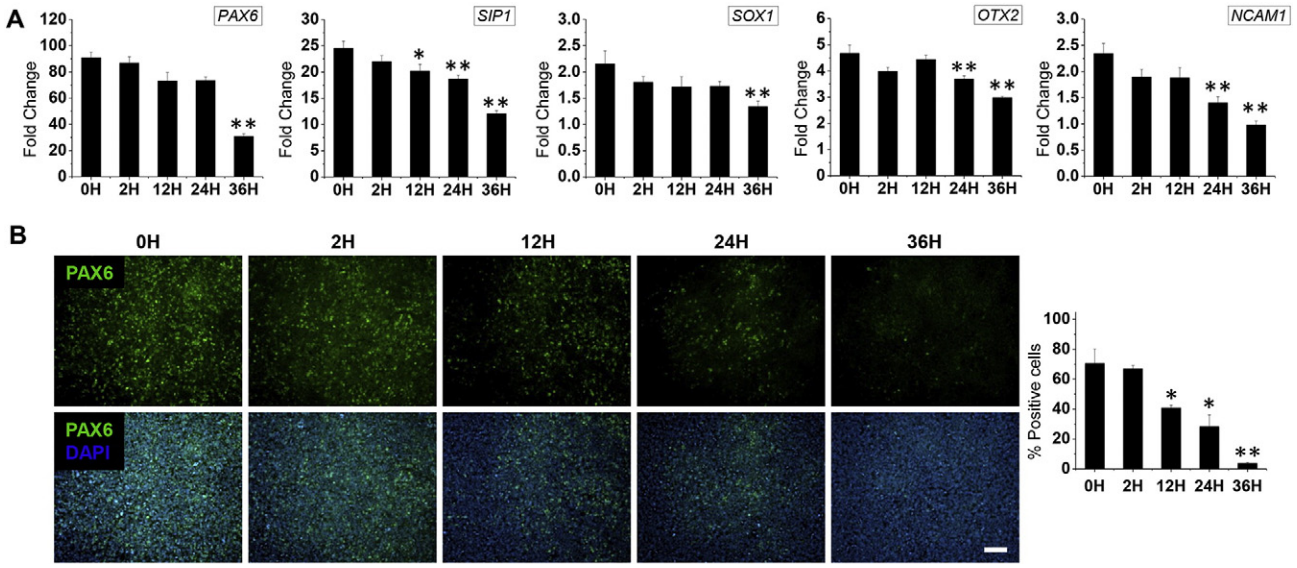

Fig. 4. Ectodermal differentiation is inhibited by the application of Y-27632 during the pre-culture period in an exposure duration-dependent manner. Human IPSCs were pre-cultured for 36 h in mTeSR™1 media (supplemented with 40 μM Y-27632 for the designated durations such as 0, 2, 12, 24 and 36 h (Fig. 2A)) followed by subsequent dorsomorphin and retinoic acid-mediated ectodermal differentiation for 72 h. (A) Gene expression of ectodermal markers *PAX6*, *SIP1*, *SOX1*, *OTX2*, and *NCAM1* was inversely correlated to Y-27632 exposure duration ($n = 6$, 3 biological samples with 2 technical duplicates, *: $p < 0.05$ and **: $p < 0.01$ as compared to 0H control. Gene expression fold change values were normalized to that of hIPSCs not subjected to either Y-27632 or differentiation). (B) Immunofluorescent imaging and its quantitative analysis showed that PAX6 expression is inversely correlated to Y-27632 exposure duration (green: PAX6, blue: DAPI, scale bar: 100 μm; $n = 6000$ cells, *: $p < 0.05$ and **: $p < 0.01$ as compared to 0H control).

3.3. Morphological modulation of hIPSCs with Y-27632 enhances mesendodermal differentiation, but not ectodermal differentiation, in an exposure duration-dependent manner

Based on the observed close relationship between cell morphology and the specification of differentiation in various adult stem cells types (McBeath et al., 2004), we hypothesized that the morphological changes of hIPSCs by Y-27632 would bias the differentiation potential of the cells. To examine the effects of Y-27632 on the differentiation potential of hIPSCs, cells were pre-cultured for 36 h with various exposure durations to Y-27632 prior to the start of mesendodermal differentiation by Activin A and WNT3A (Fig. 2A). No significant differences in expression of mesendodermal markers *T* and *MIXL1* were observed for the 2 h pre-culture with Y-27632 (Fig. 3A). In contrast, *T* and *MIXL1* were significantly enhanced by pre-culture with Y-27632 for longer exposure times of 12, 24, and 36 h. Markers of both endodermal (*SOX17* and *FOXA2*) and mesodermal (*EOMES* and *NODAL*) lineage were also significantly upregulated with 36 h exposure to Y-27632. Similarly, the expression of BRACHYURY at the protein level was also significantly increased with longer Y-27632 exposure durations (Fig. 3B). Quantitative analysis of protein expression revealed a 61% increase with 12 h-long application of Y-27632 and up to a 63% increase with 36 h. Surprisingly, the opposite trend was revealed when the cells were subjected to dorsomorphin and retinoic acid-mediated ectodermal differentiation; a decrease in expression of ectodermal markers *PAX6*, *SIP1*, *SOX1*, *OTX2*, and *NCAM1* were observed with increasing exposure duration of Y-27632 (Fig. 4A). Protein expression of PAX6 was also significantly decreased by longer exposure to Y-27632 with the 36H exposure samples exhibiting a 66% decrease in percent-positive cells (Fig. 4B). Similarly, the differentiation potential of hESCs followed similar trends to hIPSCs where mesendodermal differentiation was significantly enhanced with 36H Y-27632 exposure while ectodermal differentiation was inhibited (Fig. S4). Overall, the morphological modulation of human PSCs, by ROCK inhibitor, primes the cells to preferentially differentiate towards mesendodermal lineage.

Many labs utilize differentiation protocols which require the formation of embryoid bodies (EBs) to enhance differentiation of pluripotent stem cells via increased cell-cell contacts (Ader and Tanaka, 2014). Although it can simulate natural embryonic development, the formation of EBs to promote differentiation can be time and labor intensive. More significantly, it often results in heterogeneous and inefficient differentiation due to growth factor/nutrient diffusion limitations in EBs. Monolayer culture-based differentiation, such as protocols used in this study, has its own limitations resulting in relatively decreased efficiency in some cases, but it improves many technical problems associated with the EB-based differentiation process. In this regard, our results demonstrate a novel use of a small molecule like Y-27632 to differentially regulate cell cytoskeletal and cell-cell junctional protein organization in hIPSC monolayer culture. More importantly, such a morphological modulation provides a means to effectively enhance lineage-specific differentiation. Future studies will focus on defining the mechanistic details by which Y-27632 switches the self-renewal state of pluripotent stem cells to the primed state.

4. Conclusion

In this study, we report a previously unexplored field of interest where Y-27632 is identified as a potent small molecule to prime hIPSCs to selectively differentiate towards mesendodermal lineage. By effectively regulating the cell cytoskeleton and cell-cell junction proteins, Y-27632 induces hIPSCs to undergo EMT-like changes which predispose the cells to differentiate towards mesendodermal lineage. Simultaneously, such a disruption of actin and E-cadherin organization results in an inhibition of ectodermal differentiation. These results present a new methodology to enhance the directed differentiation of human PSCs towards mesendodermal target cell types.

Acknowledgments

This project was supported by the UCR Initial Complement Fund and the National Science Foundation (NSF) GRFP grant award number DGE-1326120. This work was also supported by the UCR Stem Cell Core Facility, funded by CIRM. The PAX6 monoclonal antibody developed by Atsushi Kawakami was obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA 52242.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2016.07.009.

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