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Hyaluronan Elevates Cell Cycle Regulators P130, E2F4, and P27[kip1] in Dormant Human Mesenchymal Stem Cells to Regulate Cell Quiescence

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Abstract

Quiescence conserves the properties of stem cells whilst they are dormant. The mechanisms associated with dormancy involve various cell regulators. Here, the effects of hyaluronan (HA; a polysaccharide associated with the extracellular matrix), on the mesenchymal stem cell cycle and the involvement of p130 and E2F family proteins in mesenchymal stem cell cycle regulation, were evaluated. Placenta-derived mesenchymal stem cells (PDMSCs) were cultured on either a normal tissue culture surface (TCS) or a surface coated with HA. Cell population percentages at the G0/G1, S, and G2/M phases were analyzed 36, 40, 44, 48, and 50 h post-seeding using flow cytometry. At 36 h, HA-treated cells had more PDMSCs in the G0/G1 phase (61.59% versus 43.61% in the TCS group). At and 48 and 50 h for the HA and TCS groups, respectively, PDMSCs had highly similar population distributions in cell cycle phases; thus, these cells were used to study cell cycle regulators. Pocket proteins (p130, p107, and pRb), E2F family proteins (E2F3, E2F4, and E2F5), using sand the binding of p130 to E2F4 and DP1 were analyzed with western blots and co-immunoprecipitation. The HA group at 48 h exhibited increased p130 and E2F4 protein expression compared with the TCS group at 50 h (4.3-fold and 1.5-fold, respectively), and the binding of p130 to E2F4 and DP1 was detected 36, 48, and 50 h post-seeding. In this studyHence, a the HA-regulated formation of a p130/E2F4 complex was observed in PDMSCs, which provides to suggests that the mesenchymal stem cell cycle is regulated by HA as an extracellular matrix.

Abbreviations: CDK: Cyclin-dependent Kinases; ECM: Extracellular Matrix; HA: Hyaluronan; PDMSC: Placenta-derived Mesenchymal Stem Cell; RB: Retinoblastoma; shRNA: Short Hairpin RNA; TCS: Tissue Culture Surface

Introduction

Stem cells possess the capacity for self-renewal, differentiation, and to act as precursors for somatic cells [1]. In humans, stem cells can be classified as embryonic or adult, which are further classified into specific types such as hematopoietic, mesenchymal, neural, limbal, intestinal, and hair follicle stem cells. Most adult stem cells are multipotent [2,3]. *In vivo*, stem cells are preserved in a dormant or quiescent state for long periods until they are required for further cell differentiation [4]. Because of their favorable properties, which include self-renewal and a lack of immune responsiveness, stem cells are a major source for cell-based tissue engineering and regenerative medicine [5].

Stem cells reside in the microenvironment known as the stem cell niche, which provides stem cell growth factors, extracellular matrices (ECMs), and niche cells for structural support and signaling transmission [6,7]. Hyaluronan (HA) is a major component of both the hematopoietic and mesenchymal stem cell niche, and is widely distributed in the connective, epithelial, and neural tissues [8]. HA is produced by hyaluronan synthase genes (*HAS1*, *HAS2*, and *HAS3*) [9-11]. HA have a linear conformation with non-sulfated glycosaminoglycans composed of D-glucuronic acid (1-β-3) and N-Acetyl-D-glucosamine (1-β-4) repeats [12,13]. HAS genes produce HAs with various molecular weights during development and extrinsic stimuli [10] and HAs are classified into 15 types according to the number of these repeats [14]. Furthermore, it regulates intracellular signal transduction via its receptor CD44 and Receptor for HA-Mediated Motility (RHAMM) [15,16]. HA has been shown to support and protect cells from damage, stress and oxidants and influence cell proliferation, differentiation, and migration [17,18]. Despite the apparent importance of HA, its influence on the cell cycle regulation of stem cells dormancy maintaining has not been comprehensively studied. *In vivo*, stem cells can be awakened from the G0 phase to enter cell cycle progression [4]. Our previous studies indicated that HA had the potential to prevent cellular senescence (an irreversible cell cycle arrest), to maintain stem cell differentiation potentials in murine adipose-derived stromal cells, and to hold mesenchymal stem cells in a reversible cell cycle arrested mode [19,20]. It was shown that the retinoblastoma (RB) family proteins, also known as pocket proteins (pRb, p107, and p130), regulated cell cycle exit and entry [21]. During cell cycle arrest, RB proteins may bind to E2F members to repress transcription of genes [22], including DNA replication-related genes for G1 progression to S phase [23]. There are eight types of *E2F* genes that give rise to 10 distinct E2F proteins, and each E2F member targetstospecific pocket protein [24]. In general, un-phosphorylated RB family members bind to E2F to repress E2F-associated gene transcription and produce cell cycle arrest [24,25]. Conversely, phosphorylated RB members dissociate from *E2F*, leading

to *E2F*-related gene transcription and G1/S cell cycle progression [26]. The *E2F* members *E2F1*, *E2F2*, and *E2F3* are known to form complexes with heterodimer proteins (DP1 or DP2) and exclusively with pRb, and they repress gene transcription and cause cell cycle arrest [27,28]. During the G0 phase, two other *E2F* members, *E2F4* and *E2F5*, bind preferentially to p130/DP1 and p107/DP1, respectively, to repress gene transcription. *E2F6, E2F7*, and *E2F8* also form a complex with DP1; however, they function independently of pocket proteins in regulating cell cycle arrest [23,29]. *E2F*-repressed cell cycle genes include those that encode cyclin-dependent kinases (CDKs) and cyclins. The E2F/RB complex may be disrupted in the presence of mitogenic stimuli, which leads to the phosphorylation of RB, activation of CDKs and cyclins, and progression of the cell cycle [30]. CDK4/6 has been shown to interact with cyclin D to initiate RB phosphorylation and G1/S transition [30,31]. Furthermore, CDK2 interacts with cyclin E to promote S phase entry [32]. Recently, the possible role of E2F was extended by reports of an association with stem cell fate [26,33]. The role of the E2F/RB complex in progenitor cells is well-studied, and the ECM is known to be important for stem cell maintenance *in vivo*; however, the influence of HA as an ECM on E2F/RB has not yet been clarified.

In this study, the E2F4/RB2/DP1 complex was analyzed in placenta-derived mesenchymal stem cells (PDMSCs) cultured in the presence of HA. We found that PDMSCs cultured in the presence of HA had a higher cell population in the G0/G1 phase compared with PDMSCs cultured on a normal tissue culture surface (TCS). Where the population distribution of cell cycle phases was similar, PDMSCs cultured on a HA-coated surface exhibited increased expression of p130, *E2F4*, and *E2F5* proteins compared to with PDMSCs cultured on a normal TCS; furthermore, p130 protein was coupled with *E2F4* and DP1.

Methods and Materials

Isolation and cultivation of PDMSCs

PDMSCs were obtained from human placentas of mothers who donated to our study at the National Cheng Kung University Hospital. Handling of the full-term placentas was approved by the Institutional Review Board. After removing the amnion and decidua, the chorionic villi were minced, washed in Hank`s balanced salt solution (Sigma-Aldrich), and then digested with 200 U/ml of type II collagenase (Worthington Biochemical Corp.) in a water bath (37°C) with orbital shaking (100 rpm) for 30 min. The tissue mixture was sieved sequentially through 500- to 104- to 37-μm mesh to remove undigested fragments. Mononuclear cells were isolated by mixing with 1.073 g/cm³ Percoll (GE Healthcare), and subsequently centrifuged at $900 \times g$ for 30 min at 4°C. They were seeded at a density of 3×10^4 cells/cm² in Dulbecco's modified Eagle's medium-low glucose (Gibco) supplemented with 10% fetal bovine serum (Gibco) and 100 U/ml gentamycin, and then incubated at 37°C in 5% CO_2. After 10–14 d, colonies of PDMSCs were trypsinized and sub-cultured for expansion on a polystyrene TCS. For treatment with HA, PDMSCs were cultured on a HA-coated surface at P5 (otherwise indicated as P4+1 throughout the article). Cells in the following experiments were all assessed at P5.

Preparation of the HA-coated surface

HA solution was prepared by dissolving HA powder (MW = 1470 kDa; Lifecore) in ddH_2O (the solution was then stored at 4°C). To produce the HA treatment, the TCS was coated with the HA solution (30 μg/cm²) and allowed to dry. Subsequently, PDMSCs were seeded at 1×10^4 cells/cm² to assess the TCS and HA treatment groups.

Identification of mesenchymal stem cell surface markers

PDMSCs were harvested at P5 and stained with the following antibodies: CD9, CD13, CD29, CD44, CD45, CD73, and HLA-ABC (BioLegend); CD34, CD90, and CD105 (BD Pharmingen). The cell populations were determined by flow cytometry (FACscan; BD) and displayed as histograms. The results were analyzed using WinMDI2.9 software (Purdue University Cytometry Laboratories).

Cell cycle population distribution percentage analysis

Harvested PDMSCs were fixed in ice-cold 70% ethanol at -20°C overnight. After cells were centrifuged at $300 \times g$ and then washed three times with PBS, they were stained with propidium iodide (Sigma-Aldrich) containing 0.5% Triton X-100 at room temperature for 1 h in darkness. They were then washed and re-suspended in PBS for flow cytometric analysis. The percentage of cells in the G0/G1, S, and G2/M phases was analyzed by using ModFit LT software (Verity Software House).

Western blots

PDMSCs were lysed for 30 min in lysis buffer with 50 mM Tris pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1% Triton X-100, and proteinase inhibitor cocktail (Roche), and then spun down at 12,000 rpm for 20 min. Proteins were separated in 10-12% gradient SDS-PAGE and transferred to a PVDF membrane. Blocking was performed using 5% skim milk in TBST (20 mM Tris, 150 mM NaCl, and 0.1% Tween 20) buffer at room temperature for 1 h. The following specific antibodies were then incubated with the proteins in blocking buffer at 4°C overnight: anti-E2F3, anti-E2F4, anti-E2F5, anti-p130, anti-p107, anti-Rb, anti-DP1 (Santa Cruz Biotechnology); anti-p27[kip1], anti-p21[cip1], anti-p57[kip2], anti-p-CDK2[Thr160] (Cell Signaling Technology); and cyclin D2 (Abcam). Unconjugated antibodies were washed away with TBST buffer, proteins were subsequently incubated with secondary antibodies at 4°C for 1 h, and then they were washed once more with TBST. After the proteins were reacted with enhanced chemiluminescence solution (Millipore), LAS-4000 (GE Healthcare) was applied to determine protein expression.

Real-time PCR

Total RNA was extracted by using TRI reagent (Sigma-Aldrich), and the cDNA was synthesized with a reverse transcriptase system (Promega). The following primer pairs were mixed with cDNA and SYBR Green I master mix (Roche), and fluorescence was assessed using real-time PCR (Light Cycler 480; Roche): E2F3 (forward 5′-TTGGGCACTACAGGTAGC-3′, reverse 5′-GTAATACACAAGGTGGCGAT-3′); E2F4 (forward 5′-TGGTGAACAAGGAGGCAT-3′, reverse 5′-GGAGTGAGCTGAGGACTAT-3′); E2F5 (forward 5′-TGATCAGCAGAAGTTGTGGC-3′, reverse 5′-TGTTGCTCAGGCAGATTTTG-3′); p130 (forward 5′-TTGGGAATTTAGATGAGCGG-3′, reverse 5′- TTAGCAATAGCCTGGGTTGG-3′); p107 (forward 5′-GATCAGCTCCTCCTTTGTGC-3′, reverse 5′-AAGGAGAGAGTGGTGGAGCA-3′); and pRb (forward 5′-TGCATGGCTCTCAGATTCAC-3′, reverse 5′-CAGACAGAAGGCGTTCACAA-3′).

Co-immunoprecipitation

Protein A sepharose CL-48 (1 g; GE Healthcare) was dissolved in 1 ml PBS containing 0.5% Triton X-100 and mixed overnight with rotation. After washing three times with PBS, equal amounts of sepharose required for each treatment were incubated with primary

antibodies at 4°C for 2 h with rotation. Following the removal of unconjugated antibodies, protein lysates were added and incubated for 2 h. Finally, the sepharose-lysates mixture was collected using centrifugation at 2,000 rpm for 3 min and then re-suspended in order to perform western blotting.

Preparation of p27-knockdown PDMSCs

The short hairpin RNA (shRNA; TRCN0000009857) that interferes with gene transcription of p27 was obtained from the National RNAi Core Facility in Taiwan. The pLKO.1-puro vector that contains the U6 promoter and binds with the p27 shRNA sequence was used to form a lentivirus by co-transfection with pCMV-Δ8.91 and pMD-G using the calcium phosphate method. The viral medium was collected and subsequently used to infect the PDMSCs for 2 d. The PDMSCs were then selected by using 3 μg/ml of puromycin (MDBio). PDMSCs that survived selection were sub-cultured for expansion and examined for knockdown efficiency using western blotting.

Statistical analysis

Statistical differences were assessed by Student's t-test. $P < 0.05$ was considered statistically significant. Data were expressed as the mean ± SEM (n = 3).

Results

Surface markers were unaffected in the presence of HA

To confirm the expression of surface markers on PDMSCs, CD9, CD13, CD29, D44, CD73, CD90, CD105, HLA-ABC, CD34 and CD45 were analyzed using flow cytometry. PDMSCs were positive for D9, CD13, CD29, D44, CD73, CD90, CD105, and HLA-ABC in the TCS and HA groups (Figure 1A). Furthermore, PDMSCs were negative for CD34 and CD45 (Figure 1A).

HA maintained PDMSCs in the G0/G1 cell cycle phase

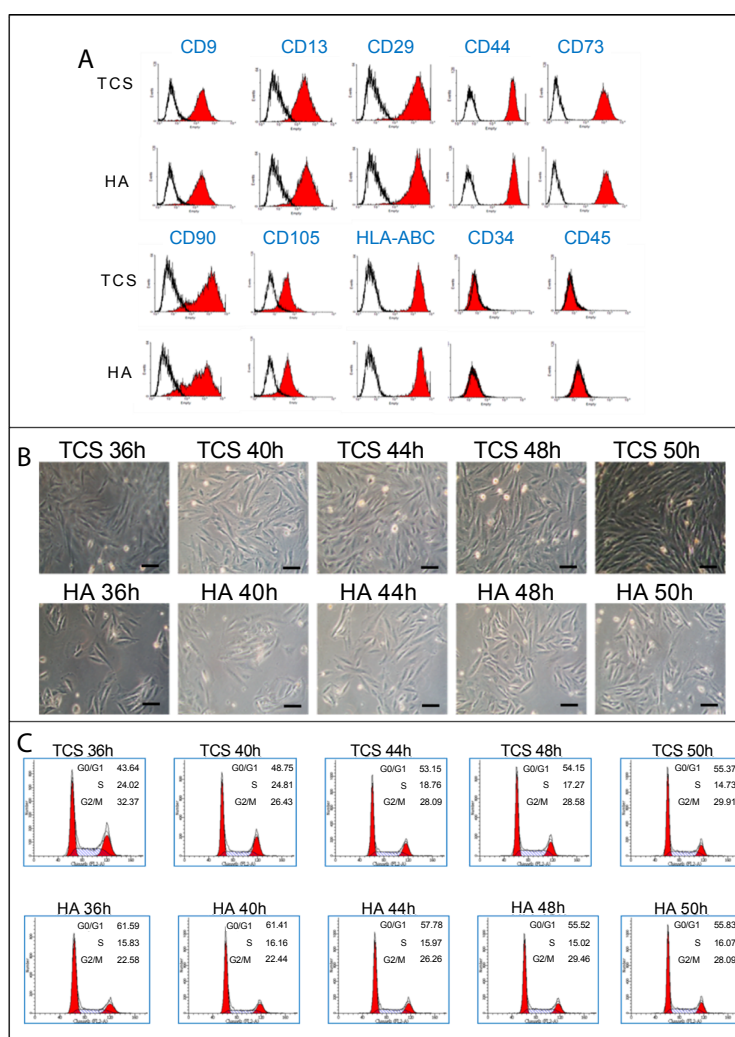The population distributions of cell cycle phases were analyzed for

Figure 1: Hyaluronan retained PDMSCs in the G0/G1 phase and maintaining stem cell marker expression. (A) PDMSCs cultured for five passages (P5 for TCS and P4+1 passage for HA) were analyzed for mesenchymal stem cells marker expression at 36, 40, 44, 48, and 50 h post-seeding. (B) The PDMSCs were seeded with equal cell density and observed at 36, 40, 44, 48, and 50 h post-seeding: cells cultured on TCS had a fibroblastic morphology, whereas HA-treatment caused PDMSCs to cluster together and show different morphology of cells. (C) PDMSCs cell cycle population distribution percentages were analyzed to determine time points with similar cell cycle population distributions. The population distribution percentages of the G0/G1, S, and G2M phases were measured using Mod Fit software. Scale bar = 100 μm.

PDMSCs to ensure that population distributions were approaching identical. Chemicals for synchronization were omitted to ensure the properties of stem cells were maintained. Hence, for the TCS and HA groups, PDMSCs at P5 and P4+1, respectively, were seeded and harvested at various time points without using synchronization chemicals. PDMSCs in the TCS group presented a fibroblastic-like morphology, whereas PDMSCs in the HA group formed clusters (Figure 1B). At 36 h, the TCS and HA groups had 43.6% and 61.6% of cells in the G0/G1 phase, respectively (Figure 1B). The population distribution among cell cycle phases was highly similar for TCS- and HA-based cells at 50 and 48 h post-cell seeding, respectively (TCS at 50 h: G0/G1 55.37%, S 14.73%, and G2/M 29.91%; HA at 48 h: G0/G1 55.52%, S 15.03%, and G2/M 29.46%). Based on these similar population distributions, the following experiments were conducted to study the cell cycle regulators (Figure 1C).

HA mediated the elevated expression of E2F4 and p130

When comparing 50 h post-seeding for TCS and 48 h for HA treatments, HA-based treated cells exhibited a 1.5-fold and 4.2-fold increase in E2F4 and p130 mRNA expression, respectively, compared with relative expression levels in TCS-based cells (Figure 2A). Additionally, the presence of HA led to increases in protein
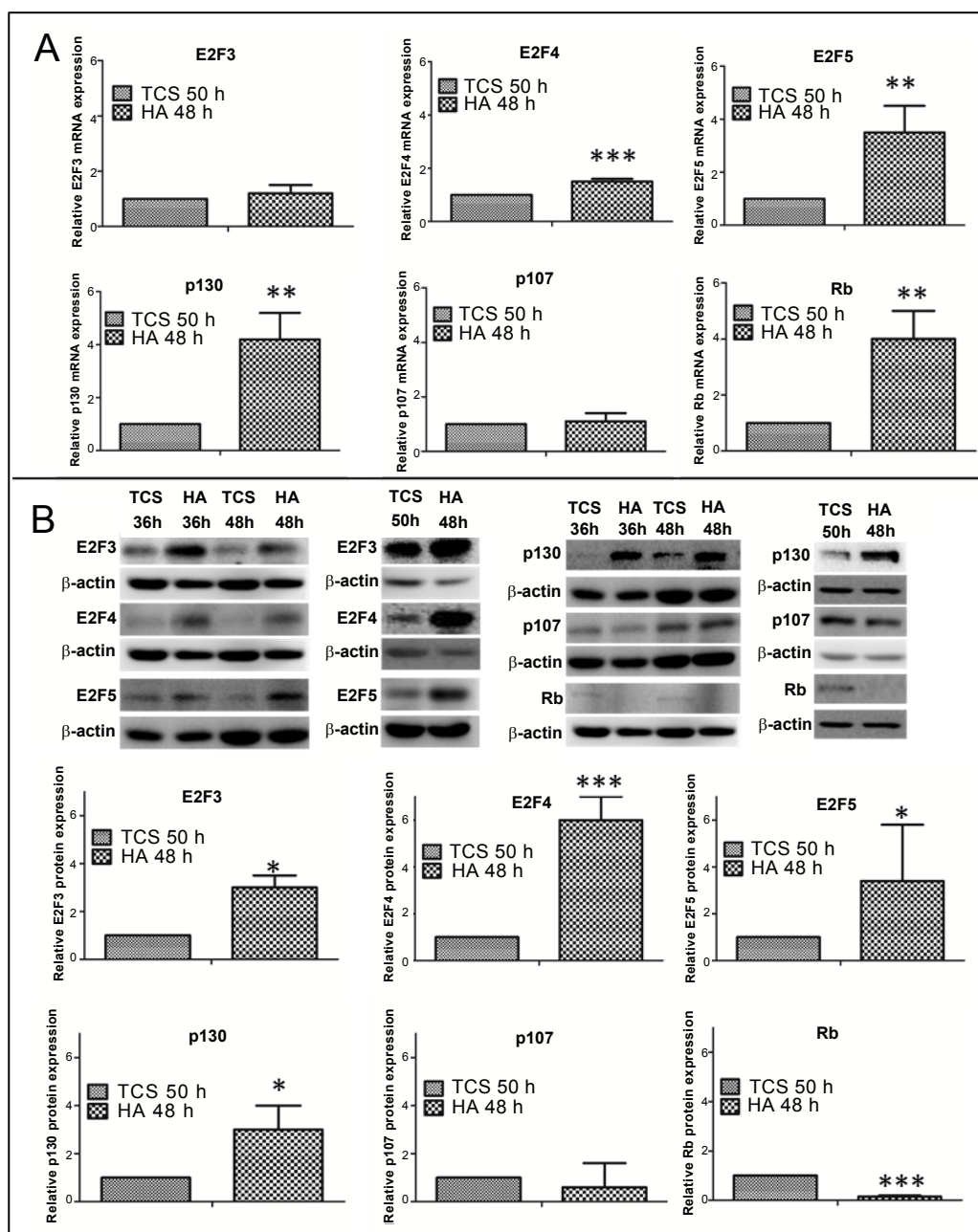

Figure 2: E2F family and p130 protein expression was HA-dependent. (**A**) The expression of E2F3, E2F4, E2F5, p130, p107, and Rb mRNA was analyzed at 50 h for TCS-based cells and 48 h for HA-treated cells. (**B**) Protein lysates were harvested at 36, 48, and 50 h for both TCS and HA groups, and western blotting was conducted to assess E2F3, E2F4, E2F5, p130, p107, and Rb. Data represent the mean ± SEM from three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus TCS.

expression as follows: 3-fold increase in E2F3, 6-fold increase in E2F4, 3.4-fold increase in E2F5, and 3-fold increase in p130 compared with the protein expression of TCS-based cells (Figure 2B). The expression of p107 protein did not differ significantly between the HA and TCS groups, whereas Rb expression was not detected in HA-treated cells (Figure 2B).

Interactions between E2F4, p130 (RB2), and DP1 were HA-dependent

Co-immunoprecipitation was performed to study the interaction between p130 and E2F4. For co-immunoprecipitation of protein lysates with p130, equal protein amounts were loaded and anti-E2F4 antibody was used to detect any coupling with p130. Results indicated that p130 formed a complex with E2F4 at 36, 48, and 50 h in the TCS and HA groups (Figure 3A). Likewise, the E2F4/p130 complex was coupled with DP1 (Figure 3B).

Transition to G1/S was blocked in the presence of HA

In HA-treated cells, the expression of p27^{kip1} was 2-fold higher and the expression of cyclin D2 was 0.6-fold lower than that relative to expression in TCS-based cells (Figure 4A). The expression of the other G1/S phase transition regulators, p57^{kip1} and p21^{cip}, did not differ significantly (Figure 4A). Based on these results, p27^{kip1} was knocked down to observe any changes in the expression of E2F4 and p130. With p27^{kip1} knockdown, expression of E2F4 decreased in HA-treated cells. Figure 4B, but levels of p130 did not decrease relative to those in the TCS group (Figure 4B). Furthermore, p130 up-levels in p27^{kip1} knockdown cell indicated the compensation effects of p130 to block G1 to S transition.

The culture conditions to which PDMSCs were exposed, i.e., growth on a TCS or on a surface coated with 30 µg/cm² HA, led to a significant difference in cell growth rate (Figure 5A). The slowing down of cell growth with HA treatment indicated that the cell cycle of PDMSCs was undergoing regulation. Taken all together, our results suggest that HA can be considered as an ECM factor that maintains PDMSCs in a dormant state, confirming by G0 specific via up-regulation of E2F4 and p130 protein expression and p130 dephosphorylation (Figure 5B). Furthermore, the PDMSCs were not released into G1/S phase progression, which is implied by the up-regulation of p27^{kip1} in HA-treated cells (Figures 4A and 5B).

Discussion

High molecular weight HA has been reported to down-regulate mitogen RAC signaling and block S phase progression, whereas low molecular weight HA produced the reverse result [34]. The molecular weight of HA applied in this study was >1470 kDa; thus, it belongs to the high molecular weight category. From the cell cycle population distribution results in the present study, PDMSCs cultured on HA produced a higher percentage of cells in the G0/G1 phase than those cultured on TCS; however, the percentage of cells was similar for HA at 48 h and TCS at 50 h for TCS and HA treatments, respectively, because cells proliferated at rate that was more rapid on TCS. In addition, the expression of mesenchymal stem cell markers indicated that HA did not promote cell differentiation.

When PDMSCs had similar cell cycle population distributions among cell cycles, mRNA expression of E2F4 and p130 was higher in HA-based treated cells compared with those cultured on TCS. This result implies that E2F4 and p130 were required at this particular stage
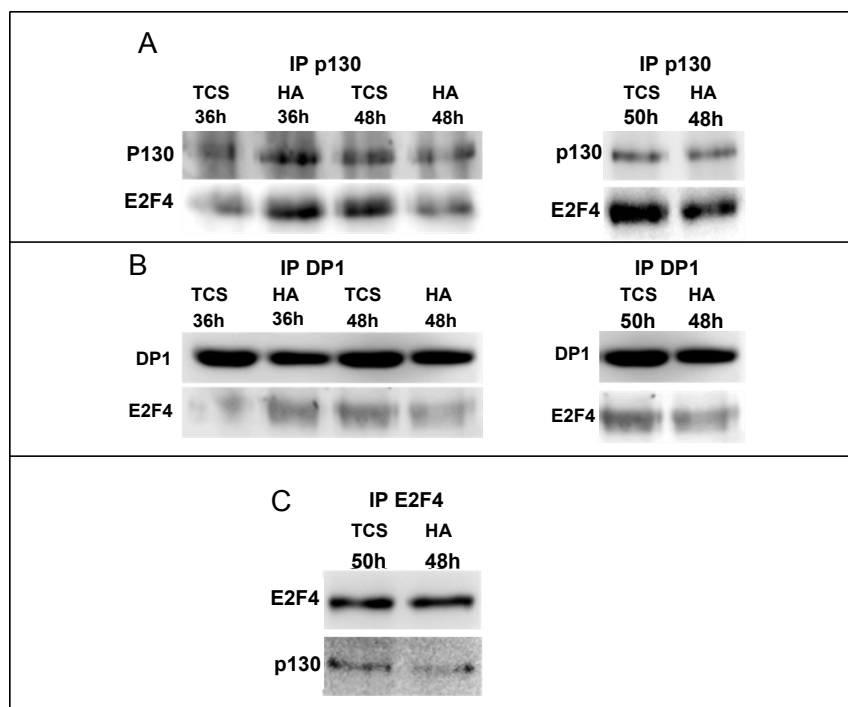

Figure 3: HA-dependent interactions between E2F4 and p130 coupled with DP1. (**A**) At 36, 48, and 50 h post-cell seeding, PDMSC lysates were harvested, co-immunoprecipitated with anti-p130 antibody, separated using SDS gel, and detected using anti-p130 and anti-E2F4 antibodies in western blots. (B) At 36, 48, and 50 h post-cell seeding, PDMSCs lysates were harvested, co-immunoprecipitated with anti-DP1 antibody, separated using SDS gel, and detected using anti-DP1 and anti-E2F4 antibodies for detection in western blots. (C) The interaction between p130, E2F4, and DP1 was confirmed through co-immunoprecipitation of the total protein lysates with anti-E2F4 antibody; anti-E2F4 and anti-p130 were then used for detection in western blots.
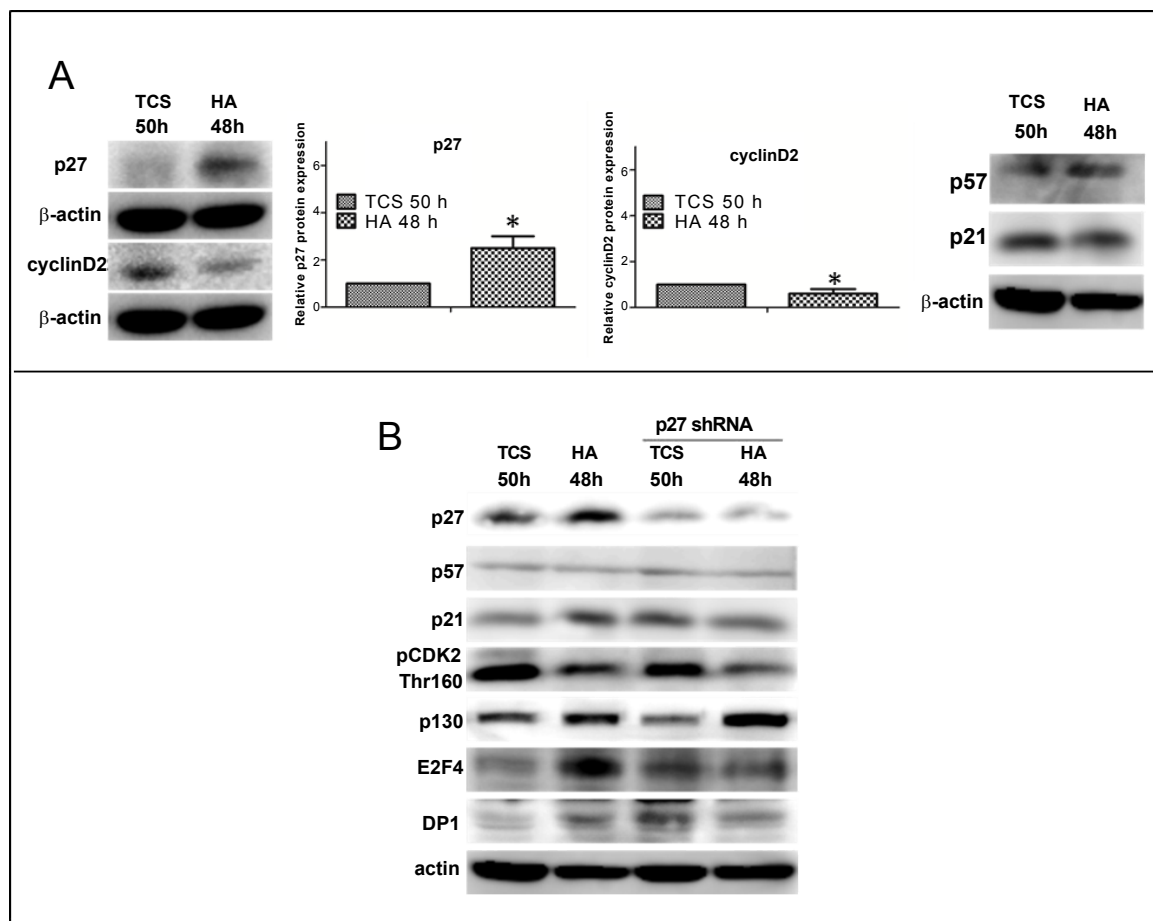
Figure 4: HA prevented transition to the G1/S phase in PDMSCs. (**A**) The G1/S transition regulators p27kip1, p57kip1, p21cip, and cyclin D2 were analyzed using western blotting at 50 h for TCS-based cells and 48 h for HA-treated cells. (B) The cells in both groups were treated with p27 shRNA to knock down p27kip1. Subsequently, protein lysates were harvested and analyzed for expression of p27kip1, p57kip1, p21cip, pCDK2, p130, E2F4, and DP1 using western blotting. The data represent the mean ± SEM from three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus TCS.

of growth, which was confirmed with western blot analysis. Previous studies showed that p130 was highly expressed compared with pRb and p107 during quiescence, and that the E2F4/p130 complex is the major contributor to E2F activity in quiescent cells [35,36]. E2F4 family proteins have been found in quiescent cells [37], which explains the up-regulation of E2F4 in PDMSCs in the presence of HA observed here. Furthermore, the stabilized E2F4/p130 complex is known to repress the transcription of genes targeted by E2F4 to slow down cell proliferation [38]. The results of the present study are consistent with previous observations of a HA-mediated slow-cell cycling phase in PDMSCs [20].

The RB family (pocket proteins) show overlapping functions [39], and the members may compensate for each other and alter their functions at different cell cycle phases [40]. A previous study discovered a complex that consisted of p130, termed the DREAM (DP, Rb-like, E2F, and MuvB) complex, which regulated cell quiescence [41]. Dual-specificity tyrosine (Y)-phosphorylation-regulated kinase 1A (DYRK1A) was reported to enhance quiescence via the DREAM complex [39]. OfConsidering the pocket proteins assessed here, the lack of Rb (i.e., pRb) expression in the HA group indicated that the pRb/E2F complex was not required in the presence of HA. Furthermore, because p107 expression was not significantly different in the HA and TCS groups, the p130/E2F4 complex was likely dominant in HA-

treated PDMSCs. Consistent with this result; G0/G1-arrested cells were regulated through the p130/E2F4 complex in a previous study [42]. Moreover, the function of p130 and E2F4 has been associated not only with quiescence [43] but also cell differentiation [44,45]. The p130/E2F4 complex is known to interact with β-catenin to activate the Wnt pathway and promote cell differentiation [45]. The RB family proteins are also important for heart development [46], and p130/E2F4 plays a role in cell proliferation during normal development [41]. However, when pRb was removed, cells were immortalized [47]; in other words, cells were completely removed from cell cycle checkpoint regulations and proliferated with a lengthened S phase. Taken together, this evidence suggests that RB proteins have a critical role to play in stem cell cycle regulation [48].

CDKs are major cell cycle regulators; cyclin D2 binds to CDK4 and CDK6, which leads to G1 phase progression from G0 [49,50]. Interestingly, cyclin D2 was previously reported to play a role in stem cell fate determination [51,52]. Consistent with this finding, in the present study, expression of cyclin D2 decreased and p27kip1 increased in HA-treated cells relative to expression in TCS-based cells. The reduction of cyclin D2 may have led to an accumulation of p27kip1 in the nucleus and decreased translocation to the cytoplasm for degradation [53]. CDK2 was reported to bind to cyclin E to promote S phase progression, whereas cyclin E and CDK2 were down-regulated
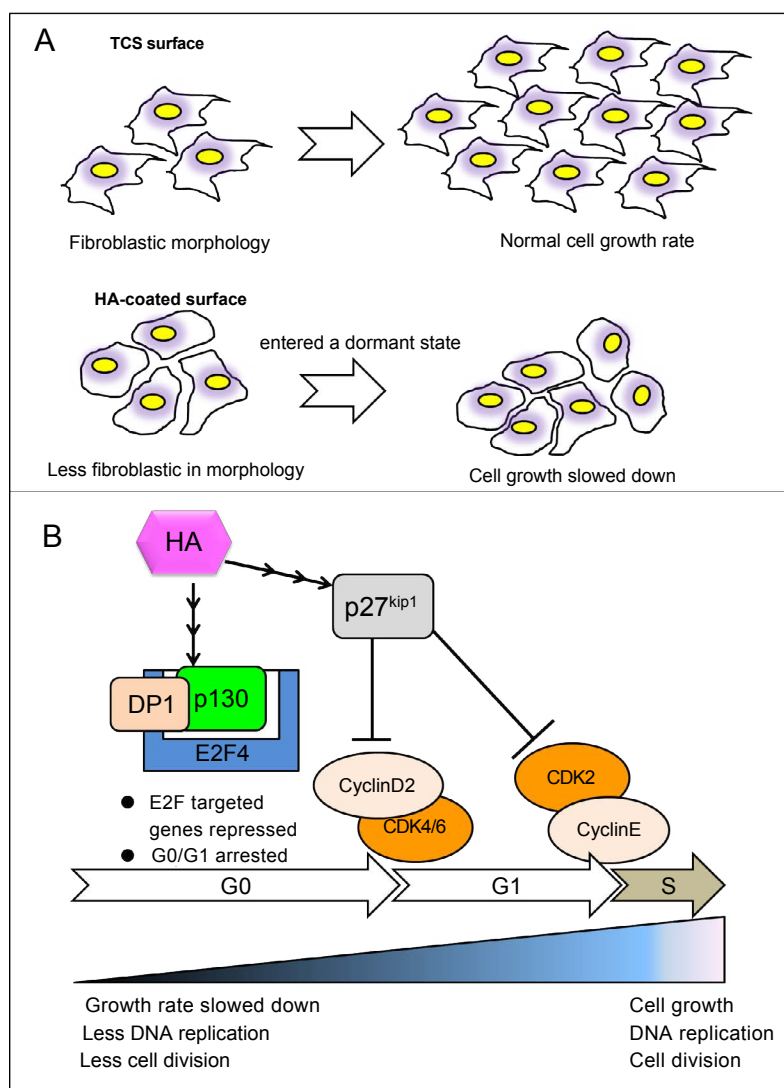
Figure 5: HA influenced the formation of the p130/E2F4/DP1 complex and the up-regulation of the G1/S transition regulator p27^{kip1}. (A) Illustration showing the condition of cell growth of PDMSCs grown on a HA-coated surface. The layer of HA somehow acted as a cushion for the PDMSCs to lie down, which triggered the PDMSCs to enter a dormant state while *in vitro*. (B) Expression of p130 and E2F4 was higher in HA-treated cells than in TCS-based cells, which suggests that p130/E2F4 complex formation was up-regulated due to the presence of HA in the surrounding cells.

by cyclin-dependent kinase inhibitors, e.g., p27^{kip1}, p57^{kip2}, and p21^{cip}, to prevent S phase progression from the G1 phase. Additionally, p27^{kip1} was found to interact with p21^{cip} to regulate quiescence in hematopoietic stem cells [54,55]. However, in the present study, the knockdown of p27^{kip1} did not lead to up-regulation of pCDK2 (the phosphorylated state of CDK2^{Thr160}) in HA-based cells, which suggests that PDMSCs were persistently maintained in growth arrest due to the presence of HA. Conversely, the loss of p27^{kip1} did not lead to down-regulation of pCDK2 in TCS-based cells, which indicates that other cell cycle regulators may have maintained pCDK2 activity to allow TCS-based cell proliferation to persist. In addition, p130 expression may have compensated for the loss of p27^{kip1} in the HA group to further prolong the cell cycle arrest in G0/G1 [56].

It is likely that the role of HA in the stem cell niche may be to maintain stem cells in their most pristine state, i.e., to allow them to lie dormant and avoid provocative stimuli. Because of the complicated state of the microenvironment, the protective action of HA towards stem cells may be readily disrupted. Prior to the present study, the involvement of HA in p130/E2F4 complex formation in mesenchymal stem cells was unclear. We conclude that p130/E2F4 was dominant in HA-treated PDMSCs, and that HA not only maintained PDMSCs in a slow proliferating state but also regulated their cell cycle through p130/E2F4 formation.

Acknowledgments

Conflict of Interest

The authors declare that there are no conflicts of interest related to this study.

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