Study of the mechanism by which Sp1/CD146 induces UCA-PSCs angiogenesis through the WNT/β-catenin pathway

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Abstract

Background

Angiogenesis is a significant factor for the cure of ischemia diseases. We found that human umbilical cord vein perivascular stem cells (UCV-PSCs) and umbilical cord artery perivascular stem cells (UCA-PSCs) have markers of mesenchymal stem cells (MSCs) and multidifferentiation abilities similar to Wharton's jelly mesenchymal stem cells (WJ-MSCs). Moreover, UCA-PSCs and UCV-PSCs have stronger angiogenesis ability and cell migration ability than WJ-MSCs, for which the regulatory mechanism remains unclear.

Results

The western blot data showed that, compared with that in WJ-MSCs, the expression of specific protein 1 (Sp1) and CD146 in UCV-PSCs and UCA-PSCs was upregulated, suggesting that Sp1 and CD146 may have an important role in angiogenesis in cell populations. By searching the GenBank promoter database, the binding site of Sp1 was found in the promoter of CD146. A proposed mechanism for angiogenesis is that Sp1 induces the UCA-PSCs angiogenesis process by activating the expression of CD146, which activates the Wnt/β-catenin signaling pathway. The β-catenin, GSK3β and p-GSK3β expression was upregulated in UCA-PSCs overexpressing CD146 and decreased in UCA-PSCs in which CD146 was silenced.

Conclusion

Our data demonstrated Sp1 activates the protein expression of CD146 in UCA-PSCs via the Wnt/β-catenin pathway mediating angiogenesis. These findings offer new ideas and strategies for the treatment of ischemic diseases.

Background

Stem cells have great potential applications in the research field of regenerative medicine [1]. MSCs, isolated from the umbilical cord (UC), adipose tissue, peripheral blood, bone marrow and other tissues, have the abilities of self-regeneration and multidirectional differentiation to be promising candidates for stem cell-based therapies [2]. The human UC is considered medical waste, and the collection of umbilical cord mesenchymal stem cells (UC-MSCs) is noninvasive and ethically noncontroversial. UC-MSCs have low immunogenicity, high proliferation capacity and the ability to secrete cytokines contributing to tissue repair [3]. UC-MSCs migrate to the site of injury and repair tissues through indirect stimulation of endogenous repair mechanisms or direct differentiation. Endogenous repair mechanisms are induced by growth factors and cytokines secreted from MSCs affecting angiogenesis, cellular migration and apoptosis [4].

WJ-MSCs derived from UC Wharton's jelly (WJ) are different from MSCs isolated from the umbilical arteries and vein [3]. There is one umbilical vein (UCV) and two umbilical arteries (UCAs) in the UC. Previous studies have shown that cells in the perivascular areas of the UCV, UCAs and in the WJ differ phenotypically from each other [5]. Several studies have demonstrated that MSCs can be isolated and cultured from several perivascular tissues [6]. Moreover, pericytes can be isolated from the perivascular area, which have the characteristics of MSCs and can form CFU-F, suggesting that the perivascular area of the UC is also the source of MSCs [7]. Pericytes express MSCs markers, have multidifferentiation ability and are the progenitors of the MSCs [8]. Pericytes are defined as cell populations expressing at least one of the following markers: neural/glial antigen 2 (NG2), αSMA, CD105 and CD146 (MCAM), without expressing CD45 and CD34 [9]. Pericytes surrounding UCAs and UCV coexpress MSC-associated markers and CD146. A previous study also showed that the increased levels of CD146 expression were positively related to angiogenic activity and cell population interactions in pericytes [10].

CD146 promotes angiogenesis by binding with vascular endothelial growth factor receptor 2 (VEGFR2) [11], which is required to activate vascular endothelial growth factor-A (VEGF-A) [12]. Additionally, CD146 has a number of ligands, such as Netrin-1, FGF-4, VEGF-C and Wnt1, that are capable of promoting angiogenesis [13]. The CD146 promoter is a GC-rich promoter, predicting including one binding element of transcription factor Sp1 and one cAMP response element binding sequence [14]. The study suggested that Sp1 interacts with the Asp element and SCA element which mediates the regulation of CD146 expression [15]. Previous studies have indicated that several pathways are activated for angiogenesis including PI3K-AKT signaling, the Wnt/β-catenin pathway, p38-MAPK signaling, Notch signaling and the Hif1a/VEGF pathway [16]. The Wnt/β-catenin pathway participated in vascular sprouting and CNS vascularization, acting by affecting angiogenic molecules expression for instance IL-8 and VEGF and controlling the proliferation, migration and differentiation of vascular cells [17]. Previous studies have shown that the CD146 expression may be related to Wnt/β-catenin activation [18]. Whether CD146 is associated with the Wnt/β-catenin pathway promoting angiogenesis through Sp1 remains unclear. In the research, we manipulated the Sp1 and CD146 expression in these three kinds of cells and explored whether Sp1/CD146 played a role in angiogenesis via the Wnt/β-catenin pathway.

Results

Characterization and differentiation of cells

Cells at the third passage morphologically resembled fibroblasts (Fig. 1a-c). These cells have multilineage differentiation potential and induce differentiation in adipocytes, osteoblasts and neurallike cells in vitro (Fig. 1d-o), which indicated that the UCV-PSCs and UCA-PSCs possessed the multipotency of MSCs, similar to WJ-MSCs.

Phenotypes of cells

Flow cytometric analysis of three kinds of cells indicated that the expression rates of the cell surface antigens CD29, CD73 and CD90 were higher than 95%, and the percentage of the immunophenotype in

the three kinds of cells for CD45, CD34 and HLA-DR was lower than 5% (Fig. 2 a-r). The results were corresponding to previous studies about MSCs surface markers [3].

UCA-PSCs exhibited better angiogenesis capacity

The ability of angiogenesis assessment in vitro was assessed by Matrigel tube formation assay. Three kinds of cells were plated on Matrigel plates, and their tubular formations were examined (Fig. 3a-c). The number of tubes in UCA-PSC (36.67 ± 3.73, n = 6) was higher than those in UCV-PSC (28.50 ± 3.10, n = 6, $p < 0.01$) and WJ-MSC (14.83 ± 3.34, n = 6, $p < 0.0001$) (Fig. 3d). Additionally, the number of branching points in UCA-PSC (38.00 ± 6.40, n = 6) and UCV-PSC (32.88 ± 3.33, n = 6) were higher than that in WJ-MSC group (15 ± 2.18, n = 6, $p < 0.0001$) (Fig. 3e). And the total tube length of UCA-PSC was longer than that of UCV-PSC ($p < 0.01$) and twice that of WJ-MSC ($p < 0.001$) (Fig. 3f). The results indicated that the angiogenesis ability of UCA-PSCs was best of three kinds of cells. Moreover, to further evaluate the proangiogenic characteristics, the three kinds of cells were combined with HUVECs. Similarly, we observed that UCV-PSCs and UCA-PSCs clearly induced HUVECs to form interconnected tubules (Fig. 3g-i), which were much more abundant than those in the WJ-MSC group (UCA-PSC, 17.83 ± 3.43, n = 6, UCV-PSC, 15.33 ± 2.75, n = 6, WJ-MSC, 8.67 ± 2.29, n = 6, $p < 0.001$) (Fig. 3j). The branching points of HUVECs were also more abundant in the UCA-PSC (24.25 ± 3.52, n = 6, $p < 0.001$) and UCV-PSC (26.16 ± 5.69, n = 6, $p < 0.01$) groups than in the WJ-MSC group (16.63 ± 1.80, n = 6) (Fig. 3k). A similar result was obtained for the length of total tube length of HUVECs in all three cell groups (Fig. 3l).

UCA-PSCs possessed stronger ability to promote HUVECs migration

UCA-PSCs and UCV-PSCs possessed stronger ability to promote the migration of HUVECs than WJ-MSCs (Fig. 4a-o). The number of migrating cells in the UCA-PSC (57.20 ± 2.97, n = 6) and UCV-PSC groups (52.60 ± 1.78, n = 6) were higher than WJ-MSC group (43.00 ± 1.95, n = 6, $p < 0.01$) (Fig. 4p). Among them, adding VEGF as the positive control, the number of migrating cells in the VEGF group (61.20 ± 2.44, n = 6) was more than those in the hESC (28.40 ± 2.38, n = 6, $p < 0.0001$), UCV-PSC (52.60 ± 1.78, n = 6, $p < 0.05$) and WJ-MSC groups (43.00 ± 1.95, n = 6, $p < 0.001$). The number of migrating cells in the hESC group (28.40 ± 2.38, n = 6), as a negative control, was less than those in the UCA-PSC (57.20 ± 2.97, n = 6, $p < 0.0001$), UCV-PSC (52.60 ± 1.78, n = 6, $p < 0.0001$) and WJ-MSC groups (43.00 ± 1.95, n = 6, $p < 0.01$) (Fig. 4p).

UCA-PSCs exhibited higher expression of angiogenesis related molecules

Previous study has shown that Sp1 is beneficial to angiogenesis and cell migration [19]. Several studies have highlighted that the central role of CD146 in vascular development [20]. The Sp1 expression in UCA-PSCs was higher than that in WJ-MSCs and UCV-PSCs (Fig. 5a). The protein expression level of Sp1 in the UCA-PSCs was higher than in the UCV-PSCs ($p <0.01$) and WJ-MSCs ($p <0.0001$), and the level of Sp1 in the UCV-PSCs was higher than that in the WJ-MSCs ($p <0.0001$) (Fig. 5b). The CD146 expression in UCV-PSCs and WJ-MSCs were lower than that in UCA-PSCs ($p < 0.0001$) (Fig. 5c), which suggest that Sp1 and CD146 may interact through some relationship.

Identification of Sp1-binding site within the CD146 promoter

According to the previous studies [14], it is predicted that the promoter of CD146 contains one binding element of Sp1. It is considered that CD146 may be a target of Sp1. As observed in Fig. 6a-b, Sp1 silence in UCA-PSCs lead to decrease in Sp1 and CD146 mRNA expression. Additionally, Sp1 silence in UCA-PSCs resulted in dose-dependent decrease in both Sp1 and CD146 protein expression (Fig. 6c). We conducted CD146-Luc reporter construct (CD146-Luc) to localize the Sp1-binding site in the promoter (Fig. 6d). CD146 promoter activity was increased by overexpression of Sp1 in UCA-PSCs (Fig. 6e). In addition, ChIP-PCR was used to analysis whether the Sp1 bind to the CD146 promoter in UCA-PSCs directly. The promoter (-1162 to -1155 bp) were recovered from immunoprecipitants of the Flag-Sp1 protein, which wasn't recovered from those of the PCMV-Flag (Fig. 6f). The results showed that the Sp1 binds to the CD146 promoter binding site.

CD146 expression was correlated with the Wnt/β-catenin pathway in UCA-PSCs

As CD146 expression in UCA-PSCs was higher than that in WJ-MSCs or UCV-PSCs, we explored the molecular mechanism underlying the greater angiogenesis ability in UCA-PSCs than in WJ-MSCs or UCV-PSCs. Recently, endothelial Wnt/β-catenin signaling was necessary for angiogenesis, contributing to vascular morphogenesis and endothelial cell specification [17]. Previous studies have shown that the CD146 expression is probably associated to Wnt/β-catenin activation in a variety of cell types [18]. We found the expression of GSK3β was higher in UCA-PSCs than that in UCV-PSCs ($p < 0.001$) and WJ-MSCs ($p < 0.05$) (Fig. 7a-b). The p-GSK3β expression was higher in UCA-PSCs than that in WJ-MSCs and UCV-PSCs ($p < 0.01$) (Fig. 7c). Moreover, the expression of β-catenin was lower in WJ-MSCs than that in UCV-PSCs ($p < 0.05$) and UCA-PSCs ($p < 0.01$) (Fig. 7d). Therefore, it is speculated that CD146 may mediate Wnt/β-catenin-induced angiogenesis to promote the development of neovascularization.

We examined whether the CD146 expression was related to Wnt/β-catenin activation in UCA-PSCs. The UCA-PSCs infected with adenovirus overexpressing CD146 exhibited higher CD146, GSK3β, p-GSK3β and β-catenin protein expression than those in UCA-PSCs transfected with si-CD146 (Fig. 7e-i). The above results indicated that Wnt/β-catenin expression is closely connected to the activation of CD146 in UCA-PSCs. These results suggested that CD146 might promote angiogenesis through Sp1 via Wnt/β-catenin pathway in UCA-PSCs.

Discussion

Our study indicated that three kinds of cells expressed MSCs markers and had multidifferentiation ability. Moreover, both UCA-PSCs and UCV-PSCs are pericytes isolated from the vascular region in the UC and have characteristics of pericytes. Previous studies indicated that cells labeled with MSCs surface markers express pericyte markers at the same time and suggested that pericytes are progenitor cells of MSCs [21]. The relationship of MSCs and pericytes was further confirmed by cell sorting for pericytes, which are multipotent for adipogenic, osteogenic and neuron-like cells, consistent with the characteristics of MSCs [22]. Furthermore, MSCs generated from different tissue sources showed different sensitivities to produce

biologically active elements which reflected the tissue of origin [23]. Previous studies have shown that pericytes derived from the vascular region inhibit the formation of myofibroblasts in ischemic tissue, decrease chronic inflammatory activity in injury sites and promote the mitosis of tissue inherent progenitor cells to regenerate damaged tissues [24]. UCV-PSCs and UCA-PSCs might play an important role in regenerative medicine, given the characteristics of pericytes described above, which are different from those of WJ-MSCs.

In this research, it is found that UCA-PSCs and UCV-PSCs possessed greater tube formation ability than WJ-MSCs, suggesting that the former two cell types promote angiogenesis prior to WJ-MSCs. Moreover, the greater migration ability of UCV-PSCs and UCA-PSCs compared to WJ-MSCs showed better application potential in promoting tissue vascular. The results indicate that pericytes wrap around blood capillaries, known as Rouget cells, which are functionally significant in vessel stability because of the absence of pericytes, resulting in hemorrhage and hyperdilation of vessels [25]. However, a clinical study has described that MSCs transplantation to the ovaries of POF patients increased the number of follicles and the level of hormones without blood flow improvement in the ovaries [26], which suggested that MSCs repair damaged tissue by secreting cytokines but lack angiogenesis ability. The pericytes markers conclude NG2, α-SMA, PDGFRβ, desmin and CD146, most of which are related to the angiogenesis process [23]. Among these markers, α-SMA considered to be a marker for pathological and physiological angiogenesis sites in tissue, PDGFRβ played an important role in the expansion and diffusion of pericytes during neovascularization, and CD146 was initially considered to be a novel biomarker of angiogenesis and a component of endothelial junctions to reduce the paracellular permeability of peripheral ECs [27].

Our research revealed that the CD146 expression in WJ-MSCs was lower than that in UCA-PSCs, which was consistent with angiogenesis. In various pathophysiological conditions, angiogenesis plays an important role in vascular development and wound repair [28]. A previous study showed that several pathways were involved in the angiogenesis process, including PI3K-AKT signaling, p38-MAPK signaling, Notch signaling, the NF- kB signaling pathway, the Hif1a/VEGF pathway and Wnt/β-catenin pathway [29]. Wnt/β-catenin signaling promotes neovascularization and has an important role in angiogenesis, because it affects the migration, proliferation and differentiation of vascular cells, along with the expression of angiogenesis factors, such as interleukin-8 and VEGF [17]. The β-catenin, GSK3β and p-GSK3β expression increased in UCA-PSCs overexpressing CD146, and the β-catenin, GSK3β and p-GSK3β expression decreased in UCA-PSCs when CD146 was silenced, which suggested that there is an interaction between CD146 and Wnt/β-catenin pathway. It is shown that the expression of CD146 is possibly connected to the Wnt/β-catenin activation inducing fibrosis in a variety of cell types, in which CD146 promoted Wnt1 induced proliferation and increased the gene expression of the fibrosis process [18]. Because CD146 is a nonstandard receptor of Wnt5a that regulates cell migration, CD146 has also been reported to regulate cell migration by recruiting myosin and actin in Wnt5a treatment [18]. However, the mechanism by which CD146 interacts with Wnt/β-catenin to promote angiogenesis remains unclear. A previous study indicated that the CD146 promoter starts 505 bp upstream of the first ATG, is GC-rich and contains a few consistent binding motifs distinct from the transcription factors CREB, AP-2 and Sp1 [15], suggesting that the transcriptional level of CD146 was upregulated through Sp1 during development.

In our study, the expression of Sp1 in UCA-PSCs was highest among three kinds of cells, suggesting that CD146 may upregulate its transcription level through Sp1. We identified that the promoter of CD146 contained Sp1 binding site.

Conclusion

In summary, we have identified that UCV-PSCs and UCA-PSCs have markers of MSCs and the abilities of MSCs, similar to WJ-MSCs. On the other hand, UCV-PSCs and UCA-PSCs showed better angiogenesis and cell migration capabilities than WJ-MSCs, and the Sp1, CD146, GSK3β, p-GSK3β and β-catenin expression in UCA-PSCs was higher than those in the WJ-MSCs and UCV-PSCs. This research thus offers a new strategy to treat ischemic diseases. The mechanism by which CD146 is upregulated through Sp1 to promote angiogenesis via the Wnt/β-catenin pathway remains unclear.

Materials And Methods

Culture and isolation of cells

Newborn UC were collected from full-term infants delivered by cesarean section at the Department of Obstetrics. UC sample collection was authorized by the Clinical Research Ethics Committee, Third Affiliated Hospital, Soochow University. UC samples were rinsed 3 times with PBS (GIBCO, Grand Island, NY, USA) to remove blood. One human UCV and two UCAs were mechanically removed from the UC [5]. The WJ, UCAs and UCV were dissected into 2-3 mm3 sections. The fragments were placed in a humidified environment at 37 ℃ and 5% CO2. The sections were cultured in low glucose DMEM with streptomycin (100 μg/ml, GIBCO), penicillin (100 IU/ml, GIBCO) and fetal bovine serum (FBS, 10%, GIBCO). Colonies of WJ-MSCs, UCV-PSCs and UCA-PSCs were observed after approximately 15 days. The UCA-PSCs, UCV-PSCs and WJ-MSCs were detected regularly as mycoplasma-free cells.

Flow cytometry analysis

Cell suspensions were incubated with various phycoerythrin (PE)-conjugated antibodies against CD34 (BD Pharmingen, San Diego, CA) and CD90 (eBioscience, San Diego, CA), an Allophycocyanin (APC)-conjugated antibody against human CD45 (eBioscience), and fluorescein isothiocyanate (FITC)-conjugated antibodies against human CD29 (eBioscience), CD73 (BD Pharmingen) and HLA-DR (eBioscience).

Differentiation of cells

The multipotency of three kinds of cells was evaluated by osteogenesis, adipogenesis and neuroid differentiation experiments. The three kinds of cells were cultured with adipogenesis induction medium (GIBCO) to promote adipogenesis differentiation. Two weeks later, oil red staining (Sigma, Steinheim, Germany) showed intracellular lipid droplets. The three kinds of cells were treated with osteogenic induction medium (GIBCO) to induce osteogenesis. After four weeks, the three kinds of cells were stained

with alizarin (Sigma) to detect the calcified extracellular matrix in the cells. For neural differentiation, three kinds of cells were cultured for 24 h in pre-medium, then cultured in modified neuronal medium for one and half day. The cells were observed by immunofluorescence for NFM and NSE described as previously [3].

Tube formation assays

Ninety-six-well plates were inoculated with 1× 104 cells per well. Then, the cells were coated with liquid Matrigel (50 μl) (Cat. No. 356234, BD Matrigel Matrix, BD Biosciences, USA) in serum-free DMEM, where the liquid Matrigel was pretreated for 30 min at 37 ℃. The tubes and branching points were photographed with a microscope (Leica), and the magnification was 100x. Branching points and tube formation were then examined in 6 random fields. Using ImageJ software, the tubular length from 6 random fields at 100x magnification per well was measured and computed as the mean.

Transwell migration assay

There were five groups: WJ-MSC group, UCV-PSC group, UCA-PSC group, negative control group and positive control group. WJ-MSCs, UCV-PSCs, UCA-PSCs, VEGF (positive control) or human endometrial stromal cells (hESCs) (negative control) (1 × 106 cells/well) were seeded into the lower chamber in twenty-four-well plates with Transwell inserts (Corning, NY, USA). The cells in the lower chamber were cultured with 1300 μl of DMEM-LG medium or DMEM-F12 medium containing 10% FBS, while HUVECs (1 × 105 cells/well) in the upper chamber were cultured with serum-free medium. Cells that migrated after 24 h of coculture, which were fixed with 4% PFA, were stained with crystal violet (0.1% w/v) for 0.5 h at 37 °C. The average number of migrated cells was determined by examining 6 random fields.

Western blot

The three kinds of cells were harvested in cell lysis buffer (CST Biological Reagents Co., Ltd., MA, USA). The cell supernatant was collected after the cells were lysed for 0.5 h at 4 °C and centrifuged for 15 min at 12000 rpm. Protein concentration analysis was performed by a BCA assay (Beyotime Biotechnology, Shanghai, CN). SDS-PAGE gel electrophoresis (10%) was used to separate the same amount (25 μg) of protein. The membranes were incubated with antibodies against GSK3β (1:5000, ab93926, Abcam, Cambridge, UK), p-GSK3β (1:10000, ab75814), β-catenin (1:5000, ab32572), CD146 (1:100, ab75769), Sp1 (1:1000, Proteintech, Manchester, U.K.), GAPDH (1:10000, AP0063, Bioworld Technology, Inc., St. Louis Park, MN, USA), goat anti-rabbit (1:10000, A0545, Sigma) or goat anti-mouse antibody (1:10000, BS12478, Bioworld Technology).

Luciferase assays

The pGL3-basic luciferase reporter plasmids loaded with the CD146 (Gene ID 4162) promoter were employed in this experiment. The construction of Sp1 plasmid (Gene ID 6667) was purchased by Genscript. Pre-confluent (60%) UCA-PSCs were infected with the plasmids using the Lipo3000(Invitrogen, Tokyo, Japan). Using the Dual-Luciferase Assay kit (Promega, Madison, WI, USA) to detect the luciferase

activities. Luciferase activity was analyzed by the fluorescence microplate reader. The firefly luciferase activity was normalized to the Renilla luciferase activity to analysis transfection efficiency.

Chromatin immunoprecipitation assay

UCA-PSCs (60% confluence) were transfected with PCMV-Flag or PCMV-Flag-Sp1. After 48 h, the UCA-PSCs cells were collected for ChIP as described previously [30]. The recovered DNA using Flag beads was assessed by PCR. 2 μl DNA was used to conduct the PCR, with primers (CD146-F 5'-TTGATCAATGTGCTGGGCTG-3' and CD146-R 5'- GTCTTGGCTAGGCTGGTCTT-3').

Small interfering RNA transfection

UCA-PSCs were transfected with control siRNA, Sp1 siRNA (50 nM) or CD146 siRNA (50 nM) separately, which were purchased from Ribo Life Science Co.,Ltd., Suzhou, CN. Transfection was mediated by Lipo 3000 (Invitrogen).

Statistical analysis

The data are shown as the mean ± SEM. To assess the difference between two unpaired groups, the unpaired Student's t test was used. To evaluate the differences among more than two groups, nonparametric one-way ANOVA was performed. All statistical analyses were performed by GraphPad 8.0. $P < 0.05$ was considered statistically significant.

Declarations

Ethical approval and consent to participate

The study was approved by the Clinical Research Ethics Committee, Third Affiliated Hospital, Soochow University. The informed consent from the patients were included. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Non-applicable.

Availability of data and materials

All analyses and results we obtained from the experiments are included in this Article.

Competing interests

The authors have no interests to disclose.

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Authors' contributions

Yanjun Yang, Lihua Zhu, Changfang Yao, and Wenfeng Ye provided the design for the study. Yanjun Yang performed the experiments and wrote the manuscript. Yuan Li and Linlin Chen participated in some experiments. Chunxue Zhang edited the manuscript. All authors have approved the final manuscript.

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Figures

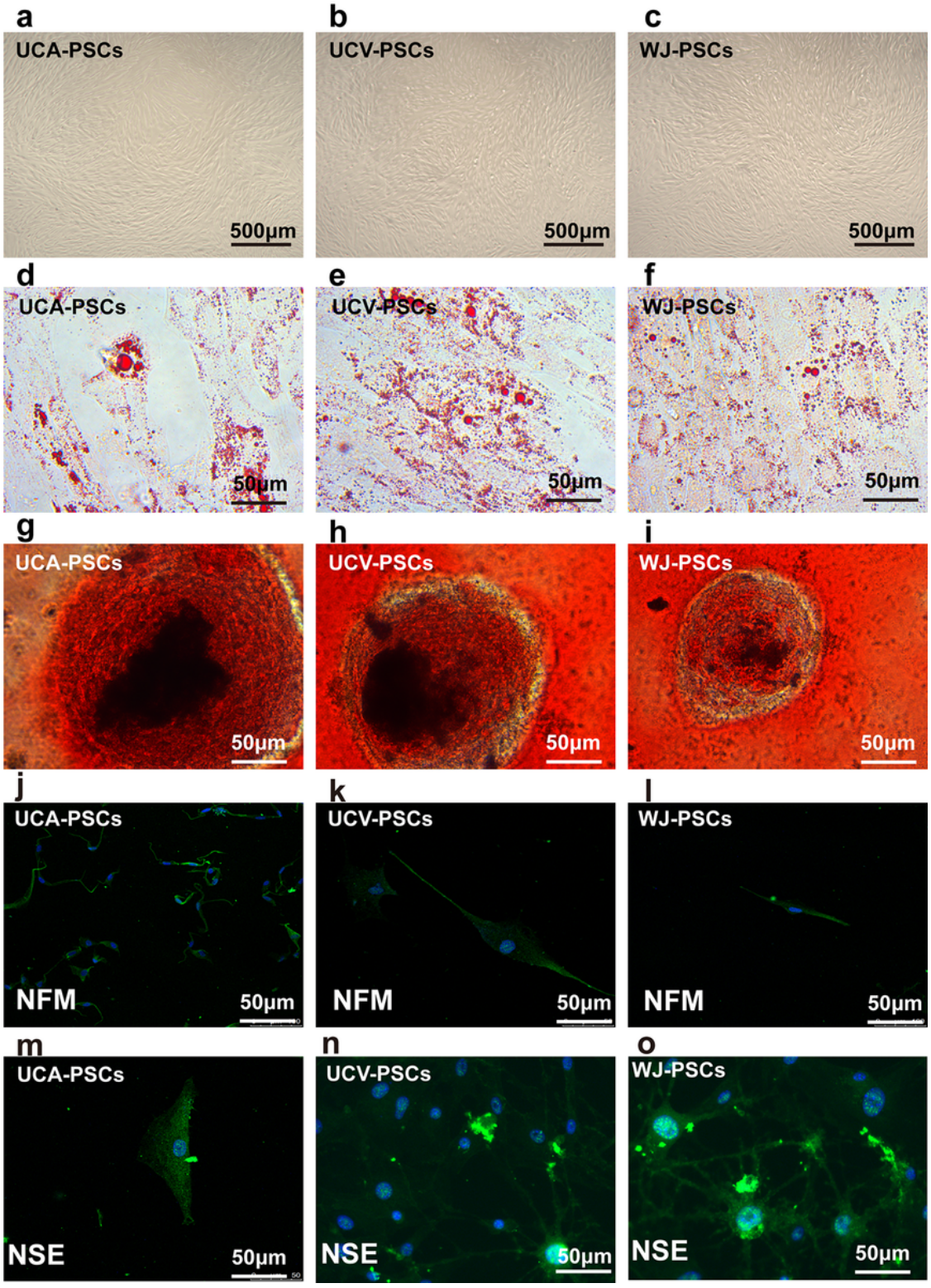

Figure 1

Characterization of cells. (a–c) Morphology of cultured UCA-PSCs, UCV-PSCs and WJ-MSCs. Bar, 500 μm. (d-f) For adipogenic differentiation, the lipid droplets in UCA-PSCs, UCV-PSCs and WJ-MSCs cultured in adipogenic induction medium were stained by Oil Red O. Bar, 50 μm. (g-i) The calcium deposition in UCA-PSCs, UCV-PSCs and WJ-MSCs cultured in osteogenic induction medium were stained by Alizarin red.

Bar, 50 μm. (j-o) The NFM and NSE in UCA-PSCs, UCV-PSCs and WJ-MSCs cultured in neurogenic induction medium. Bar, 50 μm.

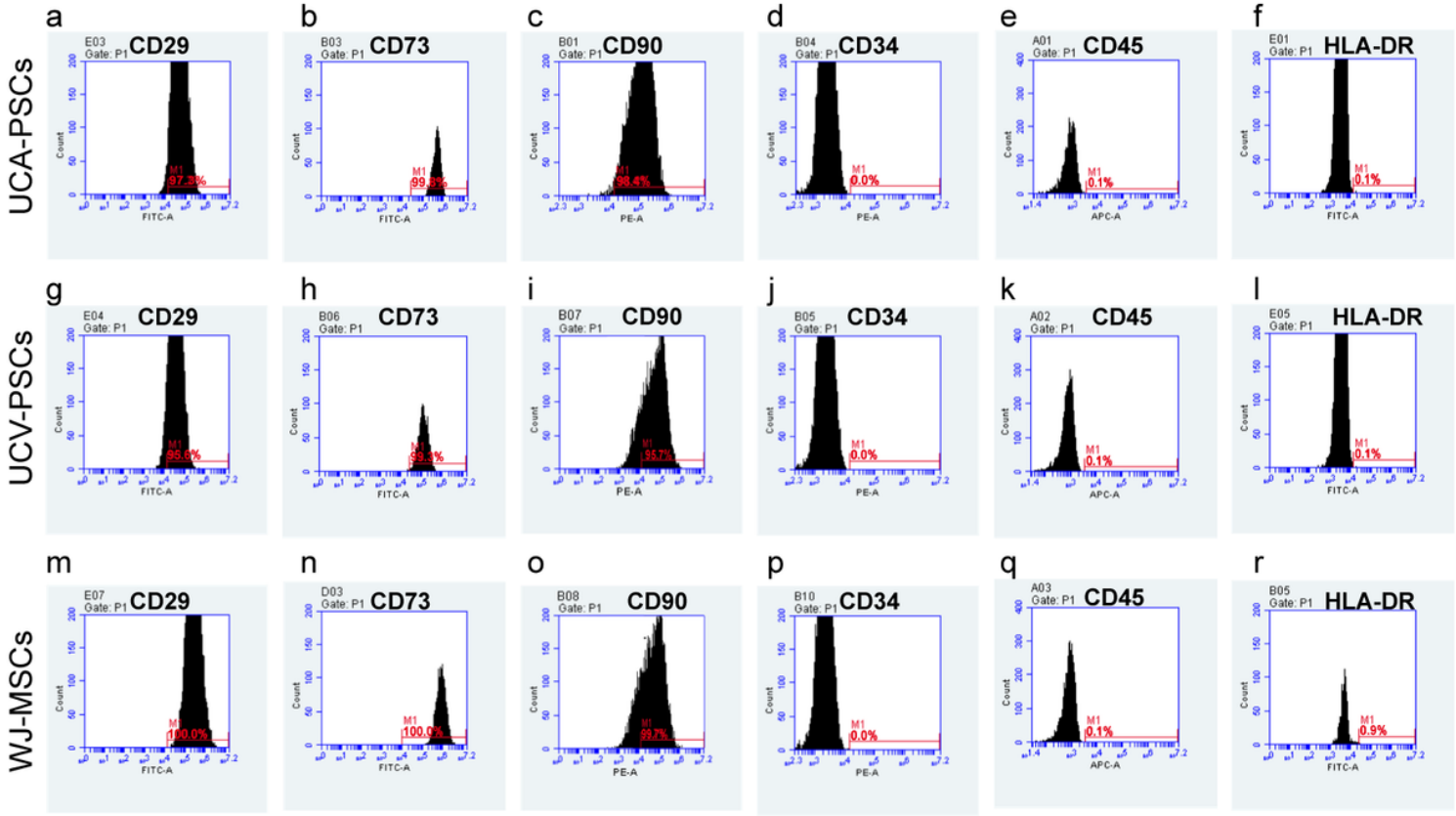

Figure 2

Flow cytometry analysis of three kinds of cells surface markers. (a-f) UCA-PSCs was positive for CD29, CD73 and CD90. UCA-PSCs was negative for CD34, CD45, and HLA-DR. (g-l) UCV-PSCs had similar markers to those of UCA-PSCs. (m-r) WJ-MSCs had similar markers to those of UCA-PSCs.

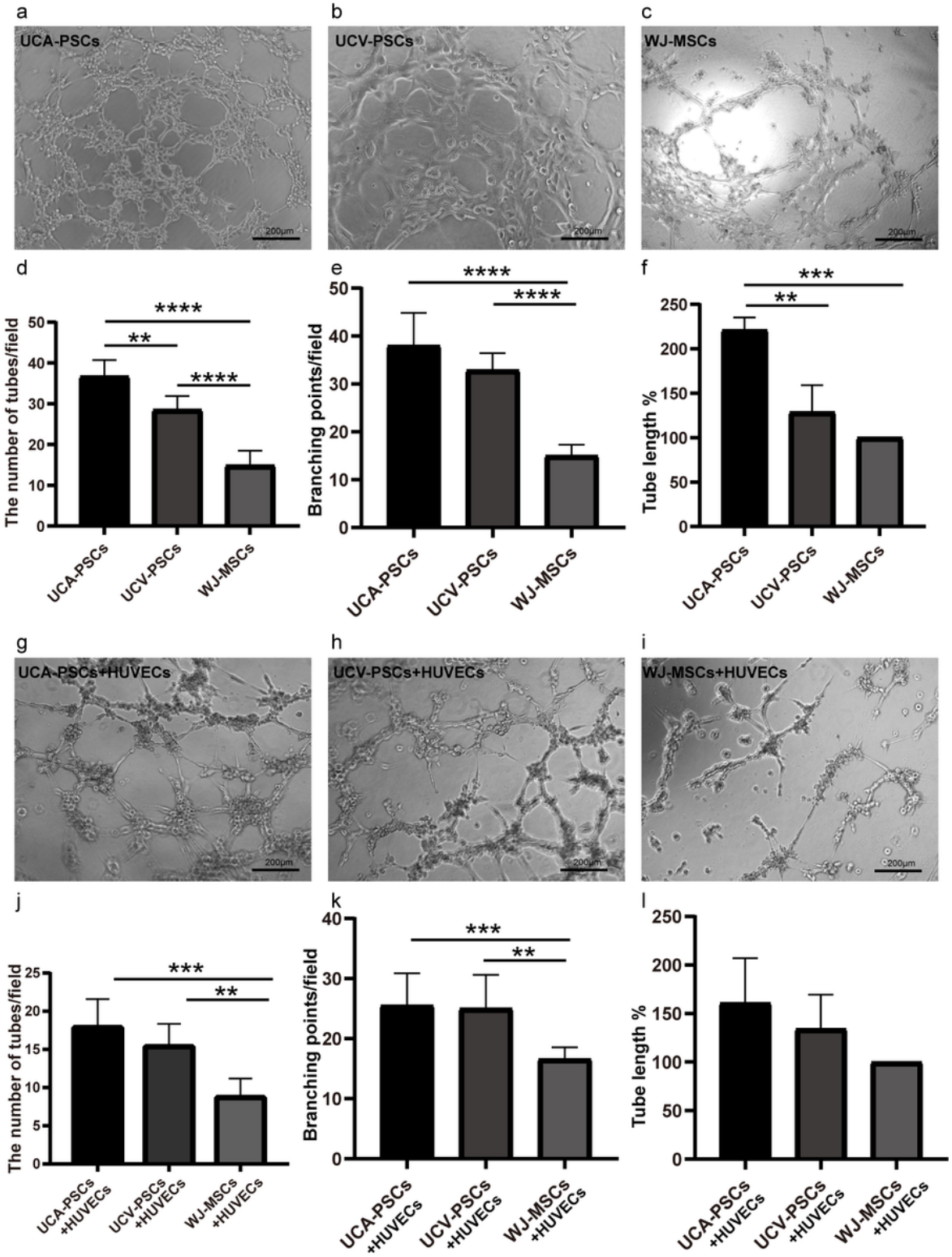

Figure 3

Tube formation assay. (a–c) Tube formation in UCA-PSCs, UCV-PSCs and WJ-MSCs was observed under microscope. Bar, 200 μm. (d-f) The number of tubes in each field in UCA-PSCs, UCV-PSCs and WJ-MSCs was counted. The branching point in each field in UCA-PSCs, UCV-PSCs and WJ-MSCs was calculated. The total length of tubes in each field in UCA-PSCs, UCV-PSCs and WJ-MSCs was quatified. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (g-i) Tube formation in UCA-PSCs, UCV-PSCs and WJ-MSCs combined with

HUVECs was observed under microscope. Bar, 200 μm. (j-l) The number of tubes in each field in UCA-PSCs, UCV-PSCs and WJ-MSCs combined with HUVECs was counted. The branching point in each field in UCA-PSCs, UCV-PSCs and WJ-MSCs combined with HUVECs was calculated. The total length of tubes in each field in UCA-PSCs, UCV-PSCs and WJ-MSCs combined with HUVECs was quatified. $^{**}P < 0.01$, $^{***}P < 0.001$.

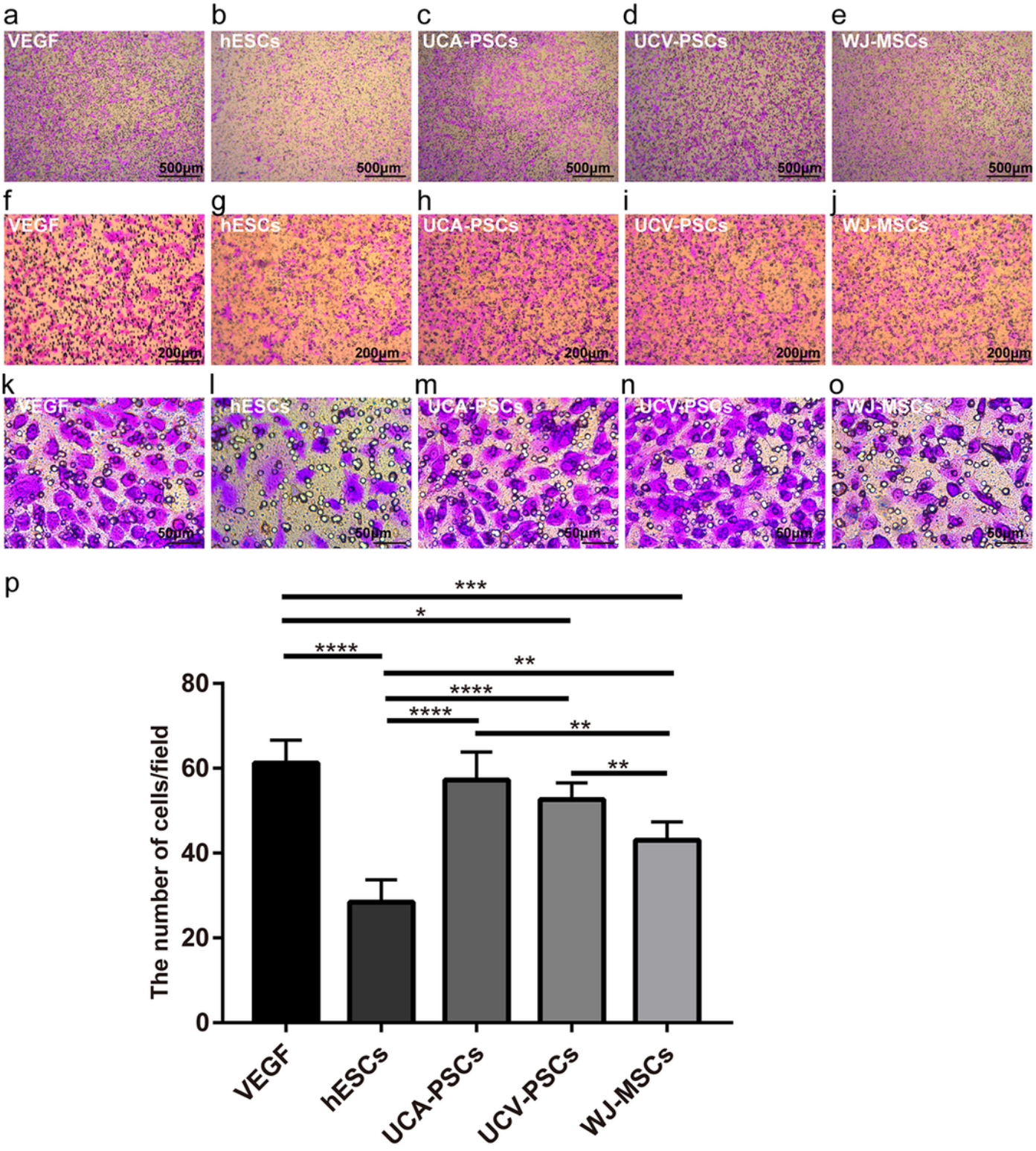

Figure 4

The Transwell Migration Assay of UCA-PSCs, UCV-PSCs and WJ-MSCs. (a-e) UCA-PSCs, UCV-PSCs and WJ-MSCs migrated during 24h. Bar: 500μm. (f-j) Three kinds of cells migrated during 24h. Bar: 200μm. (k-o) Three kinds of cells migrated during 24h. Bar: 50μm. (p) Three kinds of cells migrated during 24h. *p < 0.05, ***p < 0.001, ****p < 0.0001.

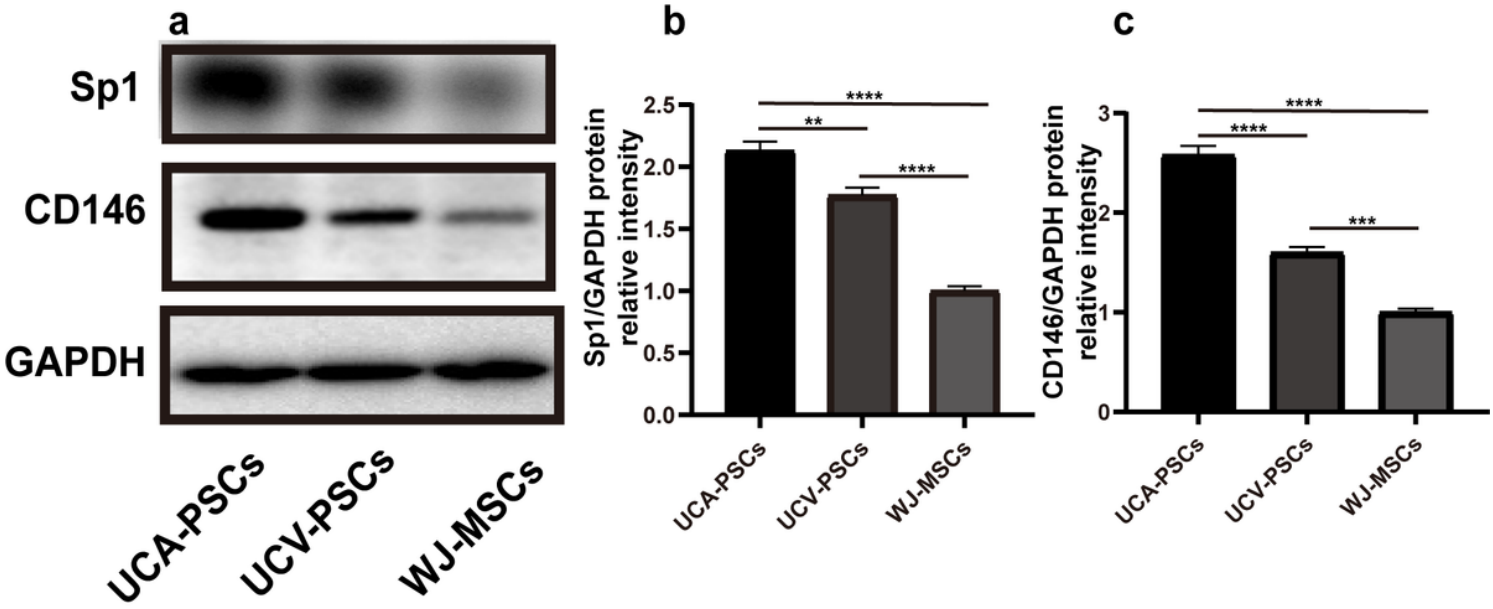

Figure 5

Expression of Sp1 and CD146 in three kinds of cells. (a) The Sp1 and CD146 expression in UCA-PSCs were higher than those in WJ-MSCs and UCV-PSCs. (b-c) Quantitative analysis of Sp1 and CD146 relative to GAPDH expression in three kinds of cells. *p < 0.05, ***p < 0.001, ****p < 0.0001.

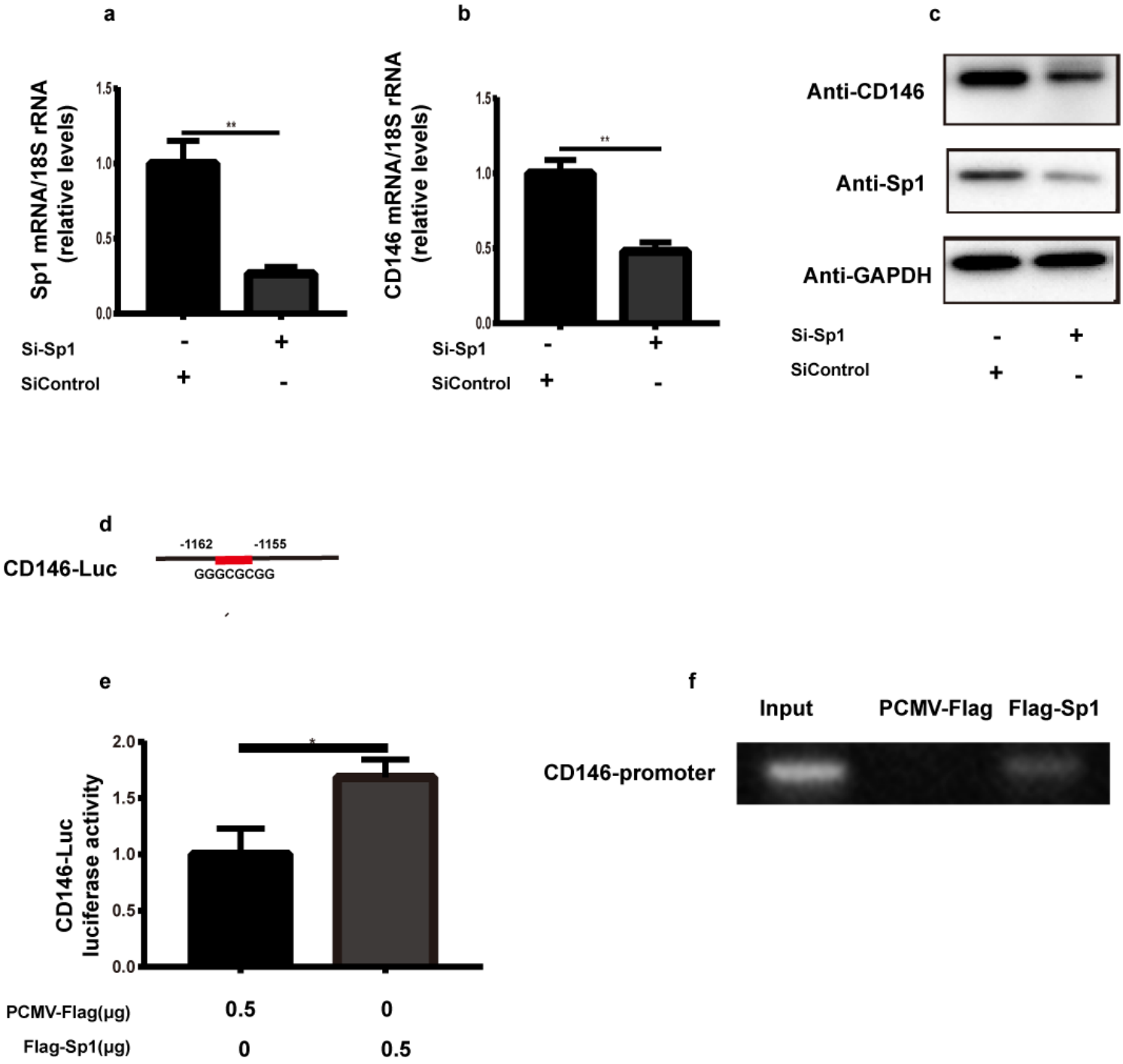

Figure 6

Verification of Sp1-binding site within the CD146 promoter. (a) The CD146 mRNA expression was increased by overexpressed Sp1 in UCA-PSCs, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (b) The CD146 protein expression was increased by overexpressed Sp1 in UCA-PSCs. (c) The CD146-Luc reporter constructs. (d) The CD146 promoter activity was increased by overexpressing Sp1 in UCA-PSCs through the luciferase reporter assay, *$p < 0.05$. (e) CHIP analysis showed that CD146 promoter is a direct target of Sp1 in UCA-PSCs.

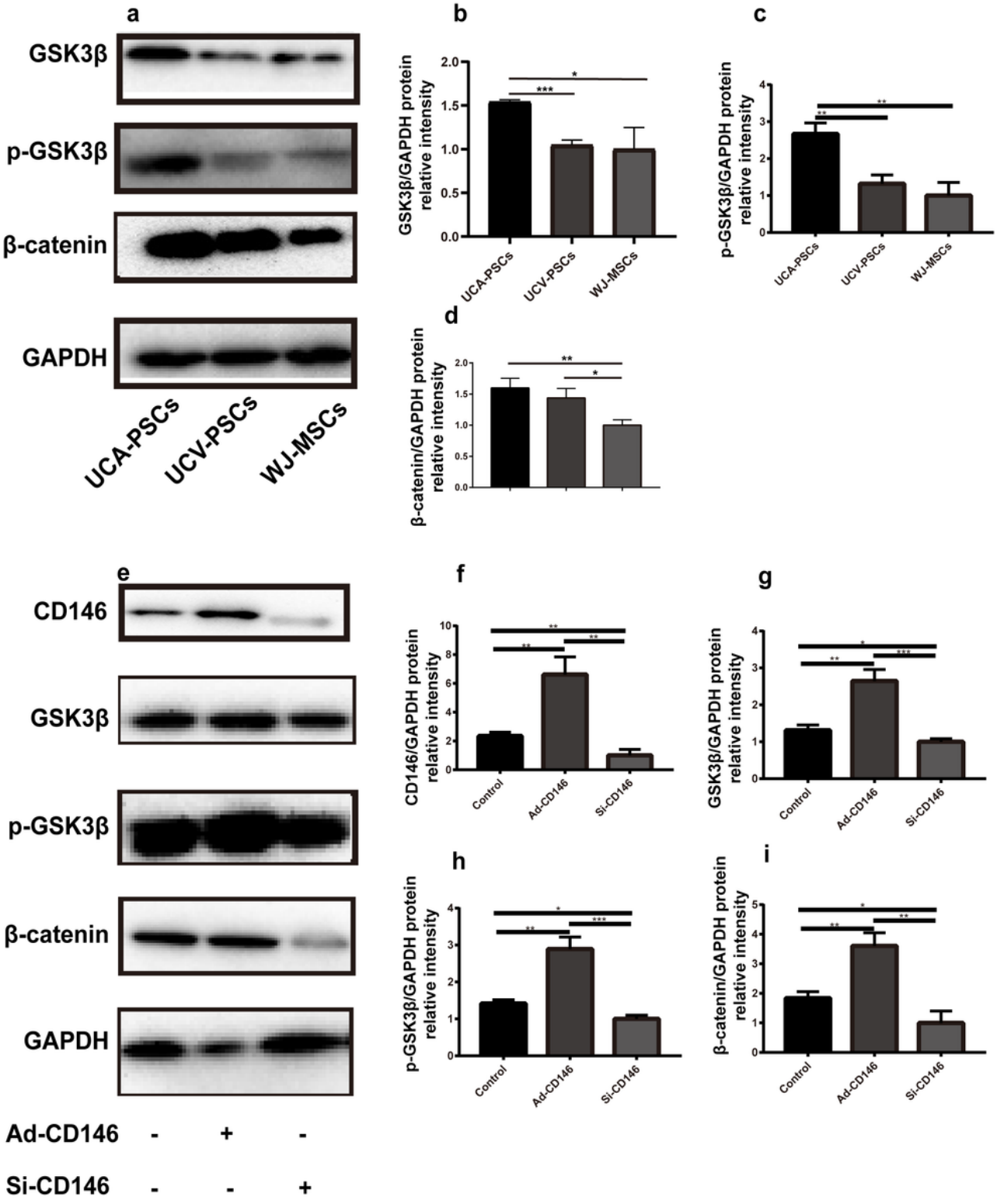

Figure 7

The CD146 expression correlated with Wnt/β-catenin pathway in UCA-PSCs. (a) The GSK3β, p-GSK3β and β-catenin expression in three kinds of cells. (b-d) The GSK3β, p-GSK3β and β-catenin expression were highest in UCA-PSCs among three kinds of cells. *p < 0.05, **p < 0.01, ***p < 0.001. (e) The CD146, GSK3β, p-GSK3β and β-catenin expression in UCA-PSCs overexpressing CD146 and UCA-PSCs silencing

CD146. (f-i) The CD146, GSK3β, p-GSK3β and β-catenin expression were higher in UCA-PSCs overexpressing CD146 than those in UCA-PSCs silencing CD146, *p < 0.05, **p < 0.01, ***p < 0.001.