YAP regulates porcine skin-derived stem cells self-renewal partly by repressing Wnt/β-catenin signaling pathway

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Research

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Abstract

Background

Skin-derived stem cells (SDSCs) are a class of adult stem cells (ASCs) that have the ability to self-renew and differentiate. The regulation mechanisms involved in the differentiation of ASCs is a hot topic. Porcine models have close similarities to humans and porcine SDSCs (pSDSCs) offer an ideal *in vitro* model to investigate human ASCs. To date, studies concerning the role of yes-associated protein (YAP) in ASCs are limited, and the mechanism of its influence on self-renewal and differentiation of ASCs remain unclear. In this paper, we explore the link between the transcriptional regulator YAP and the fate of pSDSCs.

Results

We found that YAP promotes the pluripotent state of pSDSCs by maintaining the high expression of the pluripotency genes Sox2, Oct4. The overexpression of YAP prevented the differentiation of pSDSCs and the depletion of YAP by small interfering RNA (siRNAs) suppressed the self-renewal of pSDSCs. In addition, we found that YAP regulates the fate of pSDSCs through a mechanism related to the Wnt/*β*-catenin signaling pathway. When an activator of the Wnt/*β*-catenin signaling pathway, CHIR99021, was added to pSDSCs overexpressing YAP the ability of pSDSCs to differentiate was partially restored. Conversely, when XAV939 an inhibitor of Wnt/*β*-catenin signaling pathway, was added to YAP knockdown pSDSCs a higher self-renewal ability resulted.

Conclusions

our results suggested that, YAP and the Wnt/*β*-catenin signaling pathway interact to regulate the fate of pSDSCs.

Introduction

Embryonic stem cells (ESCs) and adult stem cells (ASCs) have the ability of self-renewal and differentiation [1]. Past studies have shown that ESCs and/or ASCs derived from human, mouse and pig have the capacity to differentiate into germ cells, oocyte-like cells (OLC) and even produced offspring [2–4]. Fetal skin harbors populations of stem cells responsible for development and maintenance into adulthood, making skin an attractive source for ASCs [5]. Due to ethical considerations human embryos are not accessible for research, resulting in the use of porcine models. Porcine models have close similarities to humans with respect to their genetic relationship and the onset of epigenetic programs, making them ideal animal models for investigating human biology [6]. Porcine skin-derived stem cells (pSDSCs) offer an ideal *in vitro* model to investigate human ASCs [7]. During last 10 years, germ cells differentiated from pSDSCs have been extensively investigated [2, 5, 7]. But the efficiency of pSDSCs

differentiation to porcine primordial germ cell-like cells (pPGCLCs) was very low [7, 8]. This problem can be solved by improving the differentiation efficiency or generating large amounts of pSDSc through enhancing self-renewal. Recent studies have suggested that YAP is required for the efficient self-renewal of stem cells [9–11].

Hippo signaling pathway has been widely studied in mammalian cells and animal models [12, 13]. Hippo signaling regulates diverse cellular processes including cell proliferation and differentiation [14]. In mammals, The core components of the Hippo pathway were mammalian Ste2-like kinases (MST1/2, Hpo orthologs) and large tumor suppressor kinase 1/2 (Lats1/2, Wts orthologs). Activation of Hippo signaling results in YAP being phosphorylated, sequestered to the cytoplasm, and degraded [15]. Recently, emerging evidence suggests that YAP is a crucial regulator for maintaining the self-renewal of ESCs, induced pluripotent stem cells (iPSC), and ASCs *in vitro* and *in vivo* [9, 10, 16–18]. Additionally, YAP also regulates self-renewal of tissue-specific progenitor cells, such as skin epidermal progenitor cells, cardiac progenitor cells, muscle progenitor cells, and neural progenitor cells [19–22]. For instance, Zhang et al found that activation of the transcriptional auxiliary activity of epidermal YAP in mice can inhibit their terminal differentiation leading to the expansion of epidermal progenitor cells in the basement membrane [23].

Canonical Wnt signaling is a highly conserved pathway [24] that is dependent on *β*-catenin whose role is primarily based on whether *β*-catenin is stable in the cytoplasm or translocated to the nucleus. In the basic state, *β*-catenin in the cytoplasm is mainly inhibited by GSK3*β*, which holds Wnt signaling in an inhibited state [24, 25]. It was found that there is a multipoint cross correlation between the Wnt and Hippo signaling pathway [26]. The interaction between Hippo signaling and Wnt signaling lies in the mutual regulation of their core molecules. The Hippo signaling pathway can affect the expression of Wnt target genes by changing the transcriptional activity of *β*-catenin. Qin et al reported that YAP overexpressing hESCs display lower levels of active *β*-catenin and Wnt target genes [9]. Zhao et al found that YAP can interact with *β*-catenin and induce the expression of Wnt target genes in mouse heart tissue [27]. These studies confirmed that Hippo and Wnt signaling indeed regulate each other's activity.

Researchers suggested that the Hippo signaling pathway plays different roles in different kinds of stem cells, either being inhibitory or activating, depending on biological contexts [10, 14, 27, 28]. To date, studies concerning the role of YAP in ASCs are limited, and the mechanism of its influence on self-renewal and differentiation of ASCs remain unclear. In this study, we used the pSDSCs to explore the effect of YAP on ASCs self-renewal. Our data suggested that the upregulation of YAP contributes to the maintenance and self-renewal capacity of pSDSCs by inhibiting Wnt signalling.

Materials And Methods

pSDSCs isolation, culture and differentiation

Pigs were housed in Laixi, Shandong province. This research was approved by the Ethics Committee of Qingdao Agriculture University (Agreement No. 2019-011).

Porcine back skin tissue was collected from day 40–45 gestation fetus (E40-45). The isolation, culture and differentiation of pSDSCs were described previously [2, 7]. Briefly, the porcine skin tissue was mechanically dissociated using a pipette. The pSDSCs were incubated in SDSCs medium [7]. After 12 days culture, the cells are positive for Sox2, Oct4 and YAP (Fig. 1). These cells could differentiate into pPGCLCs under *in vitro* differentiation conditions [7]. Briefly, the pSDSCs colonies were disaggregated into single cells and 1×10^5 cells were seeded into 60 mm tissue culture dish within differentiation medium [7]. Every 2 days the differentiation medium was changed. At ~ 20 days cells with the morphological characteristics of pPGCLCs were present.

Indirect immunofluorescence assay (IFA)

Nanog, Sox2, Oct4 and YAP expression in pSDSCs were examined by IFA. The pSDSCs were collected and fixed in 4% paraformaldehyde for 30 min, then the samples were transferred to microscope slides treated with poly-L-lysine. The samples were permeabilized with 0.5% Triton-X-100 for 10 min and then blocked with 10% bovine serum albumin overnight at 4°C. The pSDSCs samples were covered with 50 µl primary antibody (Table S1) and incubated at 4°C overnight. After washing three times with PBS, the samples were incubated for 45 min at 37°C in a moisture chamber with secondary antibody (Table S1). The slides were washed three times with PBS and then incubated with Hoechst 33342 for staining the cell nucleus. Finally, the samples were viewed under a fluorescence microscope (Leica, Germany) and photographed.

Fluorescence activated cell sorting (FACS) analysis

The prepared pSDSCs (1×10^7 cells/ml) were fixed in 80% methanol. The pSDSCs were incubated successively with primary antibodies and secondary antibody (Table S1) at 37°C for 60 min. Then, the cells were centrifuged and were analyzed by Flow cytometry (Becton Dickinson, Mountain View, CA). Data were analyzed using WinMDI 2.9 software.

Small interfering RNA (siRNA) transfection

Three fluorescein-labeled siRNA targeted to YAP (siYAP-1, siYAP-2, siYAP-3) and fluorescein-labeled non-sense siRNA (siYAP-negative control, siYAP-NC) were purchased form GenePharma Co., Ltd. (Shanghai, China) (Table S2). Each siRNAs were transfected at a concentration of 30 pmol into the cells using lipofectamin 2000 (11,668 – 019; Invitrogen, Carlsbad, CA) following the manufacturer's protocol. The pSDSCs were collected for further experiment 48h post transfection. All the siRNA experiments were performed three times.

RNA extraction and RT-qPCR

As previously described [7], the total RNA was extracted from pSDSCs with the RNA prep pure Micro Kit (Aidlab, RN07, Beijing, China). The cDNA was synthesized by TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen, AT311-03, Beijing, China). RT-qPCR amplification with specific primers (Table S3) was done using a Light Cycler 480 (Roche, Germany). The relative transcript

abundance were calculated by the $2^{-\Delta\Delta Ct}$ method [29] and normalized according to the housekeeping gene *Gapdh*.

Western blot analysis

Proteins were extracted using RIPA buffer (Beyotime, P0013C, Nantong, China). SDS was mixed with the samples and boiled for 5 min in water to denature proteins, then SDS-PAGE was used to separate proteins. The proteins were transferred onto a poly-vinylidene fluoride (PVDF) membrane (Millipore, ISEQ00010, USA), and blocked in TBST containing 6% BSA for 4 hours. The membranes were incubated with primary antibody (Table S1) at 4°C, and then a horseradish peroxidase (HRP)-conjugated corresponding secondary antibody (Beyotime) was incubated at room temperature for 120 min on the second day. The BeyoECL Plus Kit (Beyotime, P0018) was used for chemiluminescence detection. The relative expression level of the target protein to GAPDH was calculated using AlphaView SA (ProteinSimple, CA, USA) software.

Statistical Analysis

GraphPad Prism 8 (GraphPad Software, San Diego, CA) was used for data analysis with One way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons; $P < 0.05$ means statistically significant difference, $P < 0.01$ means extremely significant difference, and $P > 0.05$ means not significant (ns). The data were expressed as mean ± standard error (S.E.) and all the experiments were repeated at least three times.

Results

Endogenous YAP expression in pSDSCs

pSDSCs were cultured using a previously described method [7]. Spherical aggregates of cells were clearly visible by day 8 of culture (Fig. 1A). FACS analysis during the culturing of the pSDSCs showed dynamic marker changes with the percentage positive for Sox2 being 0.09% at 4 days, 0.19% at 8 days, and 11.69% at 12 days; for Oct4, at 4 days 0.51%, 8 days 4.37%, and 12 days 27.87%; for YAP, at 4 days 5.75%, 8 days 8.76%, 12 days 41.37%. Using FACS and western blot analysis revealed that, during the culturing of pSDSCs, the percentage of the pluripotency markers Sox2 and Oct4 [30] increased dramatically. Concomitant with the increase of stem cell markers, YAP positive cells were also significantly upregulated (Fig. 1B and 1C). These results confirmed that YAP expression is increased during the culturing of pSDSCs suggesting it may play a role in self-renewal.

LPC activates YAP and promotes pSDSCs pluripotency

We explored existing data to identify a small molecule that could increase YAP expression. We found that lysophosphatidylcholine (LPC) could be hydrolyzed into lysophosphatidic acid (LPA) by autotaxin (ATX) [31] and lead to the activation of YAP in cells [32]. In order to investigate the potential effects of different concentrations of LPC on regulating YAP in pSDSCs 0, 5, 10 and 25 μM LPC was added to the pSDSCs

medium, respectively. As show in Fig. 2A, after 12 days culture, spherical colonies were observed in all treatments. FACS analysis of the pSDSCs in the various treatments showed that the percentage of Oct4 positive cells increased in the LPC-treated groups in a dose-dependent manner up to 10 μM (44.84% in 5 μM LPC, 53.79% in 10 μM LPC and 41.84% in 25 μM LPC) compared to the control group (28.67 %) (Fig. 2B). Based on these results, we used 10 μM LPC for the subsequent studies. In agreement with previous findings [31], exposure to LPC led to an increase in the mRNA and protein levels of YAP in the pSDSCs (Fig. 2C and 2D). These observations demonstrated that YAP overexpression can be induced by supplementing the culture medium with 10 μM LPC.

To investigate the potential effects of LPC induced YAP expression on regulating the self-renewal of pSDSCs, we examined the protein expression levels of Oct4 and Sox2. As shown in Fig. 2D, both Oct4 and Sox2 were upregulated, indicating that YAP plays an important role in the self-renewal of pSDSCs.

LPC induced YAP counteract pSDSCs transdifferentiated into pPGCLCs

To evaluated the importance of YAP in pSDSCs self-renewal, we examined their ability to differentiate to pPGCLCs in the presence of LPC [2, 7]. The pSDSCs were plated in differentiation medium in 6 cm dishes, and incubated for 20 days to induce the formation of pPGCLCs. In the untreated control pSDSCs formed numerous pPGCLCs and high expression of the pPGC marker Vasa (Fig. 3A and 3B); In contrast, LPC treated pSDSCs exhibited resistant to the differentiation and significantly reduced Vasa protein levels under the same differentiation conditions (Fig. 3A and 3B). Supporting this, FACS analysis found the percentage of Vasa-positive cells was 48.73% in the untreated control and 7.93% in the LPC treatment group (Fig. 3C). YAP shuttles between the cytoplasm (inactive) and the nucleus (active) [10, 14]. Therefore YAP localization is essential in maintaining the pluripotent properties of stem cells [10, 14]. In our experiments, immunofluorescent staining revealed that YAP was predominantly located in the nucleus of undifferentiated pSDSCs, while significant YAP positive signals was found in the cytoplasm of differentiated cells (Fig. 3D). Similar results to those found in a previous study using human ESCs [33]. In conclusion, these results demonstrate that YAP activation maintains pSDSCs pluripotency even under differentiation conditions *in vitro*.

Knockdown of YAP in pSDSCs by siRNA

To further confirm the role of YAP in pSDSC self-renewal and differentiation, sequence-specific siRNAs against YAP were introduced. Fluorescein-labeled non-sense siRNA (siRNA-NC) was used to optimize the transfection conditions. The expression of the red fluorescent protein was examined after transfection for 48 h by fluorescence microscope. The results in Fig. 4A demonstrating a successful transfection. In order to further investigate the transfection effect of siRNA, FACS was employed. The results showed the percentage of CY3 positive cells were similar in the siRNA-NC group and the siYAP-1 groups (Fig. 4B). In addition, RT-qPCR and western blot demonstrated that siYAP-1 had the strongest downregulation effect on YAP compared to the other three siRNA groups (Fig. 4C and 4D).

Knockdown of YAP gene expression suppressed pSDSCs pluripotency and promoted pSDSCs differentiation

Striking morphological changes were observed in the siRNA transfected group (Fig. 5A). Knockdown of YAP induced the pSDSCs cells to acquire a fibroblast-like morphology. The pSDSCs colonies lost their suspended culture and attached and spread on the culture surface. To gain an insight into the molecular mechanisms underlying this morphological phenomenon, the expression levels of two pluripotency markers, Oct4 and Sox2, and a defined terminal differentiation marker involucrin (IVL) [11] were analyzed. As shown in Fig. 5B, YAP knockdown resulted in the downregulation of Oct4 and Sox2 at the mRNA level and upregulated the expression of IVL and *β*-catenin. Western blot analysis of protein expression also indicated the same tendencies (Fig. 5C). In addition, the loss of pluripotency in the siYAP transfected pSDSCs was further confirmed by an increase in the percentage of IVL positive cells using FACS analysis. The results showed the percentage of IVL positive cells in the control group was 13.77%, but in the siYAP treated group, the percentage was increased to 24.19% (Fig. 5D).

YAP promotes pSDSCs self-renewal by suppressing the Wnt/ β -catenin signaling pathway

Previous studies demonstrated that YAP promotes human and mouse stem cell self-renewal in response to suppressing the differentiation-inducing effects of the Wnt/*β*-catenin pathway [9, 17, 30]. To elucidate the role of YAP in pSDSCs self-renewal and its connection with the Wnt/*β*-catenin signaling we investigated whether YAP regulates *β*-catenin activity. Western blot analysis revealed that the protein expression level of *β*-catenin was effectively suppressed in LPC treated pSDSCs (Fig. 2D), while *β*-catenin was robustly activated after siYAP-1 transfection (Fig. 5C). These results demonstrate a functional connection between YAP and the Wnt/*β*-catenin signaling pathway.

To further confirm the functional connection between YAP and the Wnt pathway and the role in maintaining the pluripotency of pSDSCs, we assessed the effect of the GSK3 inhibitor CHIR99021 (CHIR), which activates Wnt/*β*-catenin signaling [30], on the differentiation of pSDSCs in LPC treatment. Three dosages (1 μM, 3 μM, and 6 μM) of CHIR were used. Interestingly, increasing the concentration of CHIR in pSDSCs induced cells to acquire a flattened phenotype, indicating a loss of characteristic pSDSC morphology (Fig. 6A). FACS analysis indicated that there was a significant upregulation in the percentage of IVL positive cells in the 3 μM and 6 μM CHIR treated groups when compared with the control group (Fig. 6B). In addition, the protein expression levels of *β*-catenin and IVL were increased, but both Oct4 and Sox2 were significant decreased and the degrees of reduction of both self-renewal markers were closely correlated with the concentration of CHIR (Fig. 6C). Based on the above results, we used 6 μM CHIR for subsequent studies. As shown in Fig. 6E, the Wnt pathway was effectively repressed in the 10 μM LPC-treated pSDSCs (L-pSDSCs). Upon treating L-pSDSCs with 6 μM CHIR, the expression of *β*-catenin increased but Oct4 and Sox2 decreased (Fig. 6D and 6E). These results show that activating Wnt/*β*-catenin signaling partially restores the differentiation ability of the L-pSDSC group, as evidenced by the western blot results showing higher expression of IVL (Fig. 6E). Therefore, the results investigating the

addition with CHIR imply that YAP promotes pSDSCs self-renewal by suppressing the Wnt/*β*- catenin signaling pathway.

YAP prevents pSDSCs differentiation by modulating Wnt/ β -catenin signalling

In order to further elucidate the mechanism by which YAP regulates pSDSCs differentiation through Wnt signaling, we investigated the effect of XAV939, a Wnt signaling antagonist, on the self-renewal of pSDSCs following YAP knockdown. Three dosages of XAV939 were tested (1 μM, 5 μM, and 10 μM). With increasing concentration of XAV939 the pSDSCs showed an elevated clonal growth rate, and propagated readily in culture for more than 5 passages (data not show). Western blot analysis indicated that *β*-catenin protein was effectively suppressed in the 10 μM XAV939 treated groups but Oct4 and Sox2 were markedly increased (Fig. 7A). Moreover, FACS analysis showed that the percentage of Oct4 positive cells was significantly upregulated in the 10 μM XAV939 treatment group when compared with the control group (Fig. 7B). Next, using the siRNA system, we evaluated how YAP modulates pSDSCs self-renewal in the 10 μM XAV939 treated cells. The results indicated that siYAP led to increased expression of *β*-catenin in the pSDSCs but in the siYAP plus XAV939 group, *β*-catenin returned to the control levels (Fig. 7C). These results indicated that the Wnt pathway is important for the differentiation of pSDSCs. Moreover, it appears that YAP inhibits pSDSCs differentiation in response to Wnt/*β*-catenin signaling.

Discussion

Self-renewal is a critical aspect of stem cells allowing them to increase in number while maintaining pluripotency [19]. The molecular mechanisms involved in maintaining the self-renewal of ESCs and ASCs *in vitro* has been a key issue in stem cell research. Emerging evidence has indicated that YAP may play an important role in maintaining the pluripotency of stem cells [9, 10, 27]. To date, reports concerning the influence of YAP on the pluripotency of ASCs are limited. In this paper, we confirmed that YAP helped maintain pSDSCs ability for self-renewal through suppressing the Wnt/*β*-catenin signaling pathway. Our observations demonstrated that concomitantly with the increase of YAP positive cells, the protein levels of Oct4 and Sox2, were also significantly upregulated (Fig. 1C and 2D). On the other hand, YAP knockdown leads to a loss of pluripotency in pSDSCs (Fig. 5). Interestingly, higher expression of YAP prevented pSDSCs differentiation *in vitro* even under differentiation inducing conditions (Fig. 3A, 3B and 3C). Furthermore, we found evidence that the Wnt pathway regulates pSDSCs differentiation. Our experiments indicated that the addition of CHIR, an activator of the Wnt/*β*-catenin signaling pathway, in pSDSCs medium results in a rapid loss of stem cell properties, including morphological changes and decreased expression of the pluripotency markers, Oct4 and Sox2 (Fig. 6A and 6C). Conversely, when the Wnt/*β*-catenin signaling pathway is inhibited using XAV939 we confirmed that stem cell markers were positively upregulated (Fig. 7A and 7B). We also found evidence supporting a direct relationship between YAP and the Wnt/*β*-catenin signaling pathway. YAP overexpression or knockdown could affect the protein expression level of *β*-catenin, a core component of the Wnt pathway, and thereby inhibit or promote the differentiation of pSDSC. The results in Fig. 6D and 6E support that pSDSCs treated with LPC plus CHIR resulted in increased protein levels of *β*-catenin and IVL and decreased protein levels of Oct4 and Sox2

compared with that of LPC treatment alone (Fig. 6D and 6E). In addition, we also confirmed that knockdown of YAP would induce the differentiation of pSDSCs, but treatment with XAV939 after YAP knockdown prevented this differentiation (Fig. 7C). To summarize, these observations mutually corroborate each other and further establish that YAP plays an important role in maintaining the pluripotency of pSDSCs partly through repressing the Wnt/β-catenin signaling pathway.

Recent studies have shown that core transcription factors, such as Oct4, Sox2 and Nanog, play an essential role in establishing and controlling gene expression programs which define the identity of human and mouse ESCs [34]. In particular, these core transcription factors are important for establishing the ability of ESCs and ASCs to self-renew and differentiate [15, 35, 36]. Several studies have presented data showing that YAP can facilitate self-renewal through the upregulation of core transcription factors, such as Oct4, Sox2 and Nanog, and inhibiting YAP function results in the differentiation of ESCs [10, 16, 17]. In addition, based on transcriptional profiling, Ramalho-Santos et al demonstrated that YAP also has a critical role in the self-renewing ability of ASCs, such as neural stem cells and hematopoietic stem cells [18]. These results strongly support our work, for instance, in this paper self-renewal of the pSDSCs was identified based on the expression level of protein markers, Oct4, Sox2 and Nanog (Figure S1), and our results showed a striking correlation between the protein expression level of YAP and that of Oct4 and Sox2 (Fig. 1). YAP was highly expressed in self-renewing pSDSCs and was downregulated when cells were induced to differentiate. Meanwhile, studies described in this manuscript revealed that overexpression of YAP prevented the differentiation of pSDSCs (Fig. 2) and the depletion of YAP by siRNAs suppressed the self-renewal of pSDSCs (Fig. 5). These observations strongly confirm a particularly important role for YAP in maintaining the “stemness” of pSDSCs.

Lian et al. found that YAP can positively regulate pluripotent genes such as Nanog, Oct4 and Sox2 by genome-wide analysis of mouse ESCs [17]. Bora-Singhal et al. further found that the structural domain of the YAP protein can interact with the transcription factor Oct4 to induce the transcriptional activity of Sox2 regulated downstream targets and maintain the self-renewal ability of stem cells [37]. Reciprocally, Seo et al showed YAP expression is regulated by Sox2 and could compensate for the self-renewal defect caused by Sox2 inactivation in mesenchymal stem cells (MSCs) [15]. Given these facts, the relationship between YAP and Oct4 or Sox2 is rather interesting and complex and requires further investigation.

Extensive crosstalk between YAP and *β*-catenin, a key protein of the Wnt signaling pathway, has been observed [9, 15, 28, 38]. Qin et al. confirmed that YAP promotes human ESCs naive pluripotency by interfering with *β*-catenin and retaining it in the cytoplasm to suppress Wnt signaling.[9]. Seo et at found that in MSCs, YAP binds *β*-catenin and inhibits canonical Wnt signaling to prevent MSCs differentiation [15]. Our results are in line with these previous findings, after overexpression of YAP, the expression of *β*-catenin was decreased (Fig. 2C and 2D). Conversely, the expression of *β*-catenin increased when we knockdown YAP using RNAi (Fig. 5B and 5C). Similarly, when we added CHIR to the LPC-treated pSDSCs we found that the differentiation ability of pSDSCs was partially restored through activation of the Wnt pathway (Fig. 6D and 6E). This suggests that YAP was sufficient to maintain the self-renewal characteristics of pSDSCs by inhibiting the Wnt pathway. In order to more clearly investigate the

mechanism of YAP regulating the self-renewal of pSDSCs through the Wnt/β-catenin signaling pathway, we added the inhibitor of the Wnt pathway XAV939 to the pSDSCs that had YAP knocked down. With the addition of XAV939, we found that the cell fate of pSDSCs was altered. Compared with pSDSCs with low YAP, the addition of XAV939 resulted in pSDSCs with higher self-renewal ability (Fig. 7). Taken together, this supports YAP regulating the fate of pSDSCs though its effects on the Wnt/β-catenin signaling pathway (Fig. 8).

Conclusions

In conclusion, we have shown that YAP regulates the self-renewal of pSDSCs by inhibiting the Wnt pathway. However, the specific processes and mechanisms of the interaction between the Hippo and Wnt pathways are not fully understood. Further study of the interactions between the members of these two signaling pathways are expected to provide new insights for the molecular basis of many biological processes.

Abbreviations

SDSCs: Skin-derived stem cells; ASCs: Adult stem cells; pSDSCs: Porcine SDSCs; YAP: Yes-associated protein; siRNAs: Small interfering RNA; ESCs: Embryonic stem cells; OLC: Oocyte-like cells; pPGCLCs: Porcine primordial germ cell-like cells; MST1/2: Mammalian Ste2-like kinases; Lats1/2: Large tumor suppressor kinase 1/2; iPSC: Induced pluripotent stem cells; IFA: Indirect immunofluorescence assay; FACS: Fluorescence activated cell sorting; LPC: Lysophosphatidylcholine; LPA: Lysophosphatidic acid; ATX: autotaxin; IVL: involucrin; CHIR: CHIR99021; L-pSDSCs: LPC-treated pSDSCs; MSCs: mesenchymal stem cells;

Declarations

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Authors' contributions

SW and CSF conceived the study; YHC, SY, ZMY and SJD carried out experiments and data analysis; ZSE, Paul WD, Francesca GK and Massimo DF interpreted the data

and wrote the manuscript. All authors approved the final version.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Experiments involving live animals were conducted in accordance with the "Regulations for the Administration of Affairs Concerning Experimental Animals" promulgated by the State Science and Technology Commission of Shandong Province. The study was approved by the ethics committee of Qingdao Agricultural University.

Consent for publication

Not applicable.

Competing interests

Authors declare that no competing interests.

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Figures

Figure 1

The culture and characterization of porcine skin derived stem cells (pSDSCs). (A) The morphology of pSDSCs at different culture stages. Separation and culturing of pSDSCs and the process of passaging. Scale bar=100 μm. (B) Flow cytometry analysis of Sox2, Oct4, and YAP in pSDSCs. (C) Representative WB and relative densitometric analysis of Sox2, Oct4 and YAP expression in pSDSCs at different stages. The results are presented as mean ± SD. *P < 0.05; **P < 0.01.

Figure 2

LPC increased the expression of YAP and promoted the pluripotency of pSDSCs. (A) Morphology of pSDSCs cultured with LPC at different concentrations. Scale bar=100 μm. (B) The percentage of cells positive for Oct4 was detected by FACs of pSDSCs cultured with different concentrations of LPC. (C) Relative mRNA expression levels of ATX, YAP, Sox2, Oct4 and β-catenin in control and 10 μM treated LPC groups. (D) Representative WB and relative densitometric analysis of ATX, YAP, Sox2, Oct4 and β-catenin in control and 10 μM LPC treated groups. The results are presented as mean ± SD. *P < 0.05; **P < 0.01.

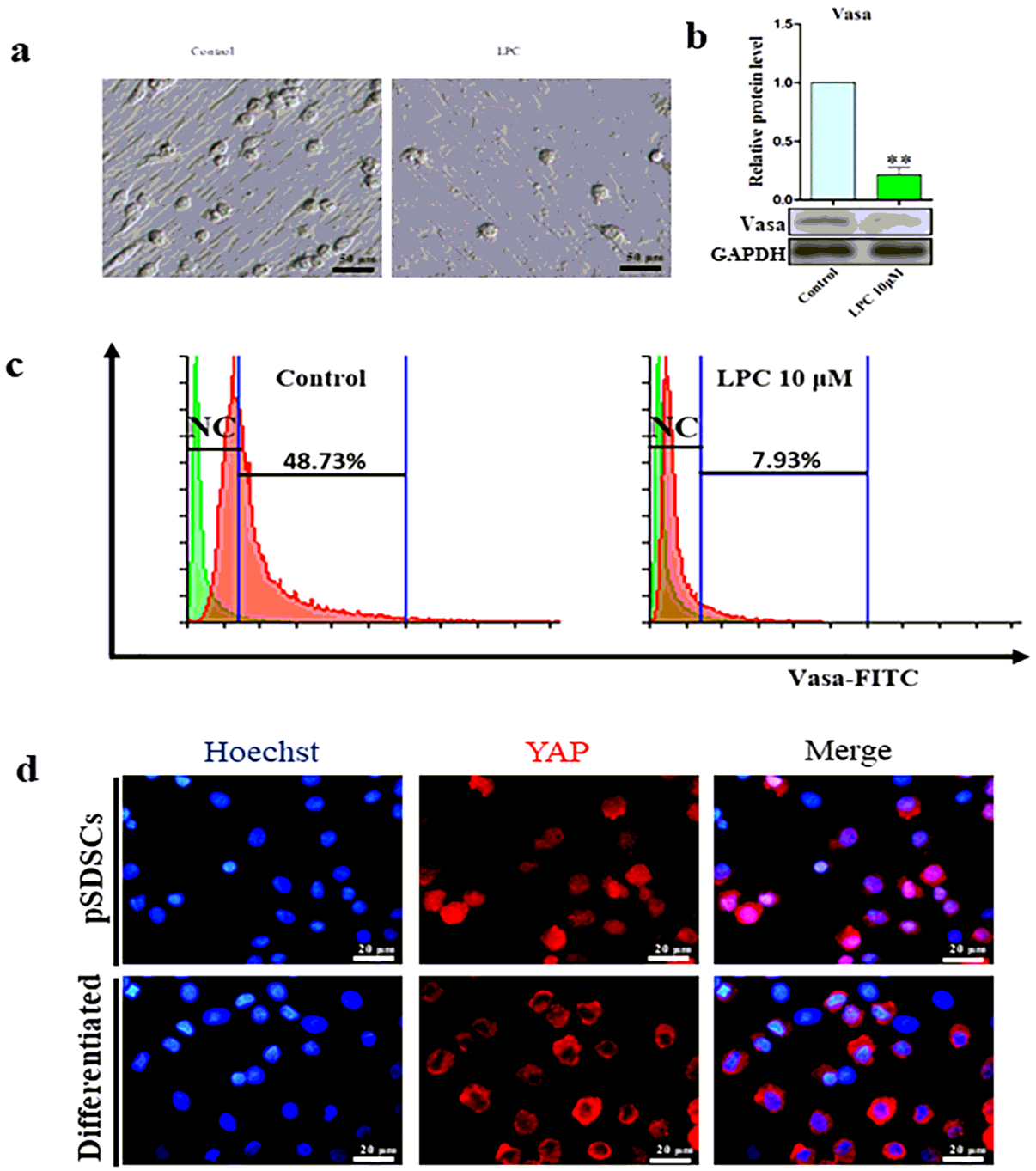

Figure 3

LPC inhibited the differentiation of pSDSCs into pPGCLCs. (A) Morphology of pPGCLCs cultured in control and with 10 μM LPC following differentiation. (B) Representative WB and relative densitometric analysis of Vasa in control and 10 μM LPC groups following differentiation. The results are presented as mean ± SD. *P < 0.05; **P < 0.01. (C) The positive rate of Vasa was detected by FACs of pPGCLCs

cultured with and without 10 μM LPC following differentiation. (D) The immunofluorescence with YAP antibody (red) and Hoechst33342 (blue) of pSDSCs and pPGCLCs.

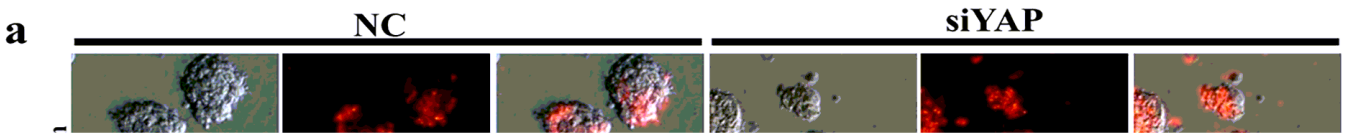

Figure 4

siYAP decreased the expression of YAP. (A) Representative image of pPGCLCs cultured with negative control (NC) and siYAP groups. (B) The positive rate of CY3 was detected by FACs of pSDSCs in control and siYAP-1 groups. (C) Relative mRNA expression level of YAP in control, NC and three YAP siRNAs

groups. (D) Representative WB and relative densitometric analysis of YAP in control, NC and three YAP siRNAs groups. The results are presented as mean ± SD. *P < 0.05; **P < 0.01.

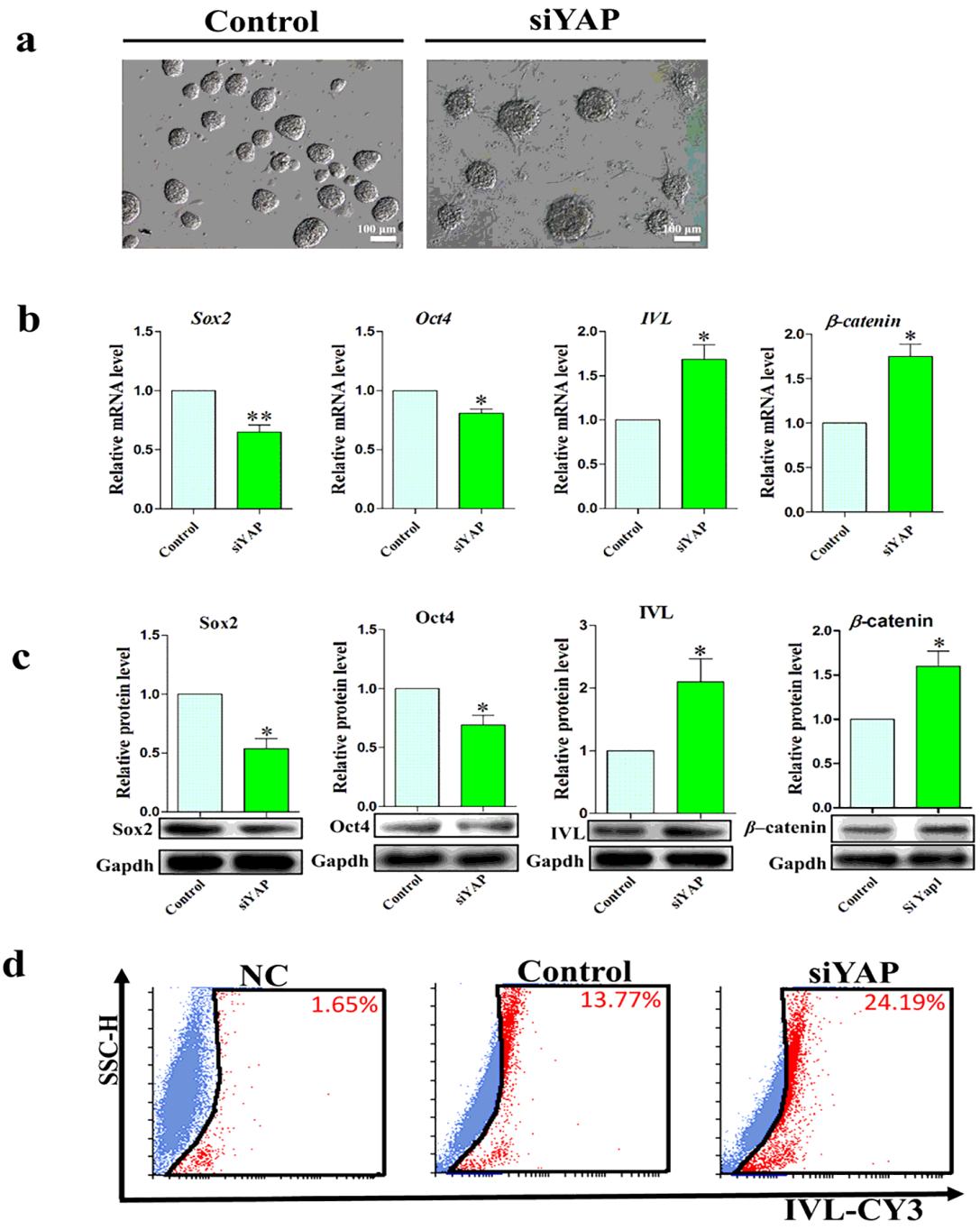

Figure 5

YAP knockdown leads to a loss of pluripotency in pSDSCs. (A) Morphology of pSDSCs with and without the knockdown of YAP. Scale bar=100 μm. (B) Relative mRNA expression level of IVL, Sox2, Oct4 and β-catenin in control and siYAP groups. (C) Representative WB and relative densitometric analysis of IVL,

Sox2, Oct4 and β-catenin in control and siYAP groups. (D) The positive rate of IVL was detected by FACs of pPGCLCs in the control, NC and siYAP groups. The results are presented as mean ± SD. *P < 0.05; **P < 0.01.

Figure 6

Analysis of pSDSCs treated with CHIR99021. (A) Morphology of pSDSCs cultured with different concentrations of CHIR. Scale bar=100 μm. (B)The positive rate of IVL was detected by FACs of pSDSCs cultured with different concentrations of CHIR. (C) Representative WB and relative densitometric analysis of β-catenin, IVL, Sox2 and Oct4 of pSDSCs cultured with different concentrations of CHIR. (D) Relative mRNA expression level of β-catenin, IVL, Sox2 and Oct4 in control, LPC and LPC+CHIR groups. E Representative WB and relative densitometric analysis of β-catenin, IVL, Sox2 and Oct4 in control, LPC and LPC+CHIR groups. The results are presented as mean ± SD. *P < 0.05; **P < 0.01.

Figure 7

Analysis of pSDSCs treated with XAV939. (A) Representative WB and relative densitometric analysis of β-catenin, Sox2 and Oct4 in pSDSCs cultured with different concentrations of XAV939. (B) The positive rate of Oct4 was detected by FACs of pPGCLCs cultured with different concentrations of XAV939. (C) Representative WB and relative densitometric analysis of β-catenin, Sox2 and Oct4 of pSDSCs in control, SiYAP and SiYAP+XAV groups. The results are presented as mean ± SD. *P < 0.05; **P < 0.01.

Figure 8

Schematic model for the mechanism of YAP regulating the fate of pSDSCs is related to the Wnt/β-catenin pathway. Overexpress YAP promotes pSDSCs self-renewal by suppressing the Wnt/β-catenin signaling pathway. Knockdown of YAP induces the differentiation of pSDSCs through activating the Wnt/β-catenin signaling pathway.

Supplementary Files

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