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ORIGINAL RESEARCH

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ICAM1 promotes the proliferation, migration, and odontoblast differentiation of human dental pulp stem cells by activating the TGF-β1/Smad pathway

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Abstract

Background: Intercellular adhesion molecule 1 (ICAM1) plays an important role in regulating cellular processes associated with tissue repair. Dental pulp stem cells (DPSCs) are essential for dental tissue regeneration, particularly in response to pulp injury. However, the regulatory mechanisms by which ICAM1 influences DPSC behavior remain to be fully elucidated. **Methods**: ICAM1 was overexpressed or silenced in DPSCs using viral transduction, and Transforming Growth Factor (TGF)-β1 expression was also knocked down to explore downstream effects. Cell proliferation was evaluated by Cell Counting Kit (CCK)-8 assay, while cell migration was evaluated via Transwell assays and Western blotting for Matrix Metalloproteinase (MMP)-2 and MMP-9. Odontoblastic differentiation was determined by Alkaline Phosphatase (ALP) activity assays, Alizarin Red S staining, and Western blot analysis of odontogenic markers including Dentin Sialophosphoprotein (DSPP), Osteocalcin (OCN), Runt-related transcription factor 2 (RUNX2), and Osterix (OSX). Activation of the TGF-β1/Mothers Against Decapentaplegic Homolog (Smad) pathway was examined through Western blotting of TGF-β1, Smad2, phosphorylated Smad2 (p-Smad2), Smad3, and phosphorylated Smad3 (p-Smad3). **Results**: Overexpression of ICAM1 enhanced DPSC proliferation, migration, and odontoblastic differentiation, which was accompanied by upregulation of the TGF-β1/Smad signaling pathway. Conversely, ICAM1 knockdown suppressed these cellular activities and attenuated pathway activation. Furthermore, TGF-β1 knockdown reversed the promotive effect of ICAM1 overexpression on odontoblastic differentiation, suggesting that ICAM1 exerts its effects in a TGF-β1-dependent manner. **Conclusions**: ICAM1 facilitates the proliferation, migration, and odontoblastic differentiation of DPSCs by activating the TGF-β1/Smad signaling pathway. These findings highlight the potential of ICAM1 as a regulatory target for enhancing dental pulp regeneration.

Keywords

ICAM1; Dental pulp stem cell; TGF-β1/Smad pathway; Odontoblastic differentiation; Proliferation; Migration

1. Introduction

The dental pulp is essential to preserving the physiological integrity of the tooth, and despite being encased within mineralized tissue, it remains susceptible to injury or microbial invasion, often leading to irreversible pulpitis or pulp necrosis [1]. In pediatric dentistry, preserving pulpal health is challenging due to the high prevalence of dental trauma and advanced carious lesions in both deciduous and immature permanent teeth, which therefore necessitate accurate assessment of pulpal status and careful selection of appropriate treatment strategies.

In recent years, treatment strategies in pediatric dentistry have shifted toward conservative and minimally invasive approaches, with a focus on preserving pulp vitality to support natural tissue repair and regeneration [2–4]. Within this context, regenerative endodontic procedures have emerged as a promising strategy, utilizing principles of tissue engineering to restore the functionality of damaged pulp tissue [5].

Dental pulp stem cells (DPSCs) were the first population of stem cells identified from dental tissue and exhibit characteristics similar to mesenchymal stem cells, including the capacity for self-renewal, high proliferative activity, and differentiation into multiple lineages [6]. When transplanted *in vivo*, DPSCs can differentiate into odontogenic, vascular, and neural cell types, contributing to the formation of tissue structures resembling the native dentin-pulp complex [7]. Given their fundamental role in maintaining pulp homeostasis and their ac-

tivation in response to tissue injury, DPSCs have been widely studied as a potential cell source for regenerative endodontic therapies [8].

Intercellular adhesion molecule-1 (ICAM1) is a member of the immunoglobulin superfamily that shares a repetitive immunoglobulin-like domain structure [9]. During enamel organ development, ICAM1 participates in diverse biological processes such as cell proliferation, differentiation, migration, apoptosis, and structural organization of tissues [10, 11]. Previous studies have shown that ICAM1 expression is regulated by microRNA-92b-5p, which modulates melatonin-induced osteogenic differentiation in bone marrow-derived mesenchymal stem cells [12]. Moreover, ICAM1 deficiency in the bone marrow microenvironment has been linked to impaired quiescence and repopulation of hematopoietic stem cells [13], highlighting its role in stem cell regulation. In addition, ICAM1 expression has been found to be upregulated in deep carious lesions, which is associated with compromised odontogenic differentiation of DPSCs and increased secretion of pro-inflammatory cytokines and chemokines [14], suggesting that ICAM1 may influence the inflammatory response and regenerative capacity of dental pulp tissue.

The transforming growth factor-β1 (TGF-β1)/Smad signaling pathway is a key regulator of osteogenic differentiation, primarily through promoting the proliferation of osteoprogenitor cells and the upregulation of downstream target genes [15]. ICAM1 has also been implicated in regulating this pathway in other biological contexts, such as promoting bone metastasis in triple-negative breast cancer [16]. Therefore, we speculate that ICAM1 may play a role in DSPC differentiation by regulating the TGF-β1/Smad pathway.

Despite these associations, the precise role of ICAM1 in DPSC biology remains poorly understood. Therefore, we designed this study to investigate the function and underlying mechanisms of ICAM1 in regulating DPSC behavior *in vitro*, with a focus on its potential interaction with the TGF-β1/Smad signaling pathway.

2. Method

2.1 Cell culture

Third-passage human DPSCs (Zhenjiang Weigan Biotechnology Co., Ltd.) were cultured in α-modified Eagle's medium (11900024, α-MEM; Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin. All cells were maintained at 37 °C in a humidified incubator containing 5% CO_2. Once the cells reached approximately 80% confluence, osteogenic induction was initiated using osteogenic differentiation medium, consisting of basal medium supplemented with 10 nM dexamethasone (D4902), 10 mM β-glycerophosphate (G9422), and 50 mg/mL vitamin C (A4403) (all from Sigma-Aldrich, St. Louis, MO, USA).

2.2 Cell transfection

DPSC cells were transfected with shRNA-ICAM1 and shRNA-TGF-β1 using Lipofectamine® 2000 reagent (11668013, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. The target sequences for shRNA-ICAM1 and shRNA-TGF-β1 were 5′-GCGCGAGGTTTTCCCGGAAAG-3′ and 5′-GCTGACAGCTTTGCGAATTAA-3′. After Polymerase Chain Reaction (PCR) amplification using pcDNA3.1, the human ICAM1 gene was directly cloned into a linearized adenoviral vector (Adeno-X Adenoviral System 3 Kit), and following confirmation through DNA electrophoresis and gene sequencing, the recombinant plasmid was transfected into linearized DPSC cells for packing. Then, the effectiveness of the infection was verified using DPSC cells infected with AdenoX-ICAM1 (ad ICAM1). All small molecules in this experiment were obtained from GenePharma (Shanghai, China). Transfection and gene expression efficiency were confirmed by Western blot analysis.

2.3 Characterization of DPSC

The phenotypic profile of the DPSCs was assessed by flow cytometry using antibodies against Thy-1 cell surface antigen (CD)90-Phycoerythrin (PE) (FAB2067P, R&D Systems, Shanghai, China) and CD105-Allophycocyanin (APC, 562408, BD Biosciences, San Jose, CA, USA), as previously described [17]. Multilineage differentiation potential was evaluated using standard staining protocols: Alizarin Red S for osteogenic differentiation, Alcian Blue for chondrogenic differentiation, and Oil Red O for adipogenic differentiation.

2.4 Cell counting Kit-8 (CCK-8)

Cell proliferation was evaluated using a CCK-8 kit (C0037, Beyotime, Shanghai, China), according to the manufacturer's protocol. Briefly, DPSCs were seeded into 96-well plates at a density of 8×10^3 cells per well. After 24 hours, 10 μL of CCK-8 reagent was added to each well, and the plates were incubated for 1 hour at 37 °C, protected from light. Absorbance was measured at 450 nm using a microplate reader to determine cell viability.

2.5 Migration assays

DPSCs (2×10^5 cells) were seeded into the upper chambers of Transwell inserts (Corning, NY, USA) containing medium with 1% fetal bovine serum (FBS). The lower chambers were filled with medium containing 10% FBS to create a chemotactic gradient. After 24 hours of incubation at 37 °C, non-migrated cells were removed from the upper surface, while migrated cells on the lower surface of the membrane were fixed and stained with 0.5% crystal violet, then imaged and quantified under a light microscope.

2.6 Alkaline phosphatase (ALP) activity assay

To evaluate early odontogenic differentiation, ALP activity was measured on day 7 using a 5-Bromo-4-Chloro-3-Indolyl Phosphate/Nitro Blue Tetrazolium (BCIP/NBT) ALP assay kit (S0026, Beyotime, Shanghai, China), following the manufacturer's protocol. Total protein concentration was determined using the bicinchoninic acid (BCA) assay (P0012, Beyotime, Shanghai, China). Absorbance was recorded at 405 nm (ALP

activity) and 562 nm (BCA assay) using a microplate reader.

2.7 Alizarin Red S (ARS) staining assay

Mineralized matrix formation was assessed by ARS staining after 21 days of odontogenic induction. Briefly, cells cultured in six-well plates were fixed with 4% paraformaldehyde for 15 minutes at room temperature and rinsed with phosphate-buffered saline (PBS), then incubated with 1% Alizarin Red S solution (G1452, Solarbio Science & Technology, Beijing, China) at 37 °C for 30 minutes. Excess dye was removed by rinsing the wells three times with deionized water at five-minute intervals. The stained cultures were air-dried prior to imaging.

2.8 Western blot analysis

Total protein was extracted from DPSCs using Radioimmunoprecipitation Assay (RIPA) lysis buffer (P0013B, Beyotime, Shanghai, China) supplemented with protease and phosphatase inhibitor cocktails. Protein concentrations were quantified with a BCA assay kit. Equal amounts of protein were separated by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) on 10% polyacrylamide gels and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA). The membranes were blocked with 5% skim milk for 1 hour at room temperature, followed by overnight incubation at 4 °C with primary antibodies. After washing three times with Tris Buffered Saline with Tween-20 (TBST), membranes were incubated with Horseradish Peroxidase (HRP)-conjugated secondary antibodies (1:3000; Cell Signaling Technology) for 1 hour at room temperature. Protein bands were visualized using an enhanced chemiluminescence detection system. The primary antibodies used in this experiment included: ICAM1 (1:1000, ab109361), MMP-2 (1:1000, ab92536), MMP-9 (1:1000, ab76003), DSPP (1:1000, ab216892), OCN (1:1000, ab133612), RUNX2 (1:1000, ab192256), OSX (1:1000, ab209484), TGF-β1 (1:1000, ab215715), Smad2 (1:1000, ab309195), p-Smad2 (1:1000, ab309196), Smad3 (1:1000, ab40854), p-Smad3 (1:1000, ab52903), and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH, 1:1000, ab8245); all from Abcam, Cambridge, UK. Band intensities were quantified using Image-Pro Plus 6.0 (Media Cybernetics, Rockville, MD, USA).

2.9 Statistical analysis

All experiments were conducted with at least three independent replicates. Data are expressed as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism (10.5.0, GraphPad Software, San Diego, CA, USA). Comparisons between two groups were analyzed using Student's *t*-test, while comparisons among multiple groups were evaluated using one-way Analysis of Variance (ANOVA). $p < 0.05$ was considered statistically significant.

3. Result

3.1 ICAM1 promotes the proliferation of DPSCs

Flow cytometry analysis confirmed that the isolated DPSCs expressed typical mesenchymal stem cell surface markers, including CD90 and CD105 (Fig. 1A). Following three weeks of induction, DPSCs exhibited successful multilineage differentiation, as evidenced by Alizarin Red S staining for mineralized nodules (Fig. 1B), Alcian Blue staining for chondrogenic matrix deposition (Fig. 1C), and Oil Red O staining for intracellular lipid accumulation (Fig. 1D). These findings confirm the osteogenic, chondrogenic, and adipogenic differentiation capacity of DPSCs.

To investigate the functional role of ICAM1, we established stable ICAM1 knockdown (sh ICAM1) and overexpression (ad ICAM1) DPSC lines via lentiviral transduction. Western blot analysis confirmed the efficient knockdown and overexpression of ICAM1 in the respective groups (Fig. 1E). CCK-8 assays revealed that ICAM1 overexpression significantly enhanced DPSC viability, whereas ICAM1 knockdown led to a marked reduction in viability (Fig. 1F). These findings indicate that ICAM1 positively regulates the proliferative capacity of DPSCs.

3.2 ICAM1 promotes the migration of DPSCs

To assess the impact of ICAM1 on DPSC migration, Transwell assays were performed. Quantitative analysis showed that ICAM1 knockdown significantly impaired the migratory ability of DPSCs, while ICAM1 overexpression enhanced migration compared to control cells (Fig. 2A). To further validate these findings, we examined the expression of matrix metalloproteinases MMP-2 and MMP-9, which are involved in extracellular matrix degradation and cell migration. Western blot analysis revealed that ICAM1 overexpression upregulated MMP-2 and MMP-9 levels, whereas ICAM1 knockdown reduced their expression (Fig. 2B). Together, these results suggest that ICAM1 promotes DPSC migration, likely by modulating MMP expression.

3.3 ICAM1 enhances the odontogenic differentiation potential of DPSCs

To evaluate the effect of ICAM1 on odontogenic differentiation, DPSCs transduced with either ad ICAM1 or sh ICAM1 were subjected to 21 days of odontogenic induction. Western blot analysis was performed to assess the expression of key differentiation markers. Overexpression of ICAM1 markedly increased the protein levels of dentin sialophosphoprotein (DSPP), osteocalcin (OCN), runt-related transcription factor 2 (RUNX2), and osterix (OSX), whereas knockdown of ICAM1 led to a reduction in their expression (Fig. 3A).

RUNX2 and OSX are transcription factors essential for initiating osteogenic differentiation. In this study, their upregulation in the ICAM1 overexpression group indicated the successful activation of the early osteogenic program. OCN, a marker of late-stage osteoblast differentiation, was also significantly upregulated, suggesting the progression to mature osteoblastic phenotypes. DSPP, a specific marker of odontoblasts, reflects

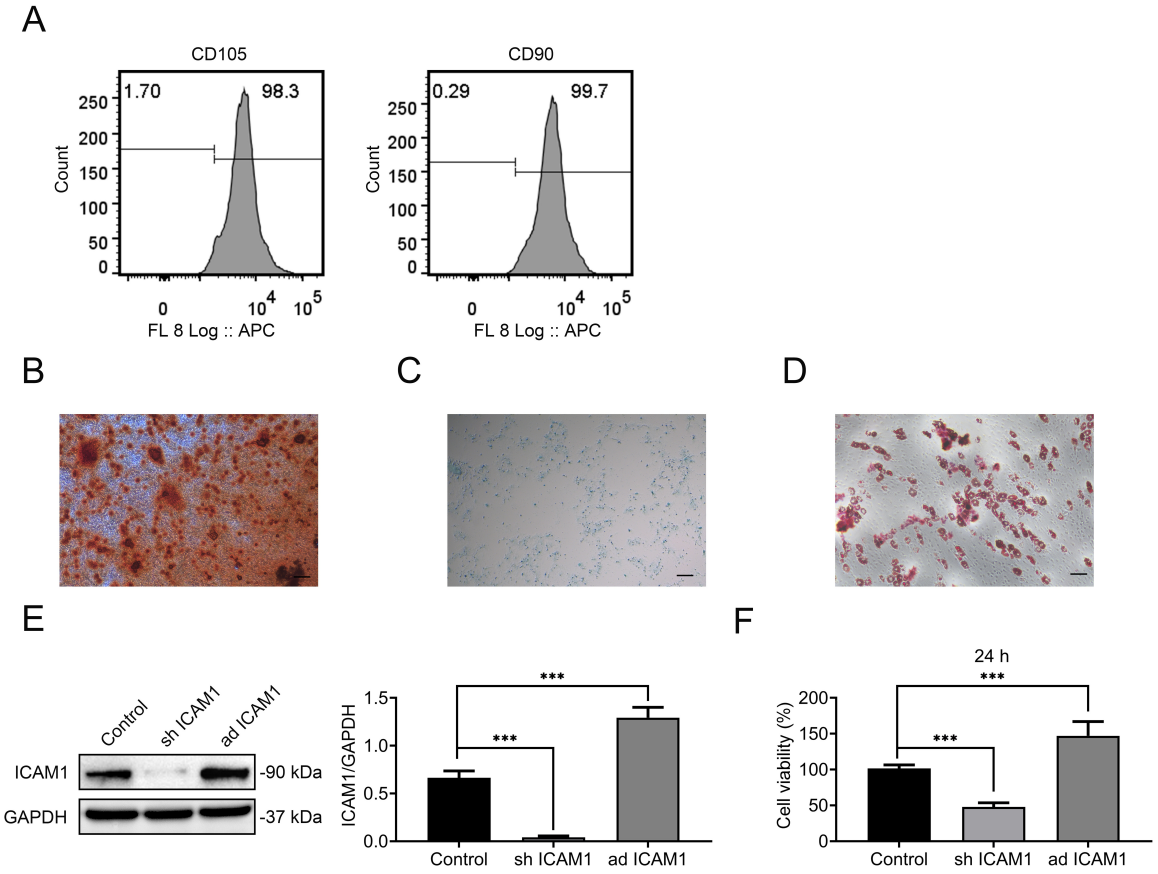

FIGURE 1. ICAM1 promotes the proliferation of DPSCs. (A) Flow cytometric analysis showing DPSC expression of mesenchymal surface markers CD105 and CD90. (B) Alizarin Red S staining revealing mineralized nodule formation following osteogenic induction. (C) Alcian Blue staining demonstrating chondrogenic matrix deposition. (D) Oil Red O staining indicating intracellular lipid droplet accumulation. (E) Western blot analysis confirming ICAM1 overexpression and knockdown in DPSCs. (F) Cell proliferation assessed by CCK-8 assay in control, ICAM1-overexpressing (ad ICAM1), and ICAM1-silenced (sh ICAM1) groups. Data are presented as mean ± SD; ***$p < 0.001$; technical replicates, n = 3. ICAM1: intercellular adhesion molecule 1; CD: Cluster of Differentiation; FL: Flight Level; APC: Allophycocyanin; GAPHD: Glyceraldehyde-3-Phosphate Dehydrogenase.

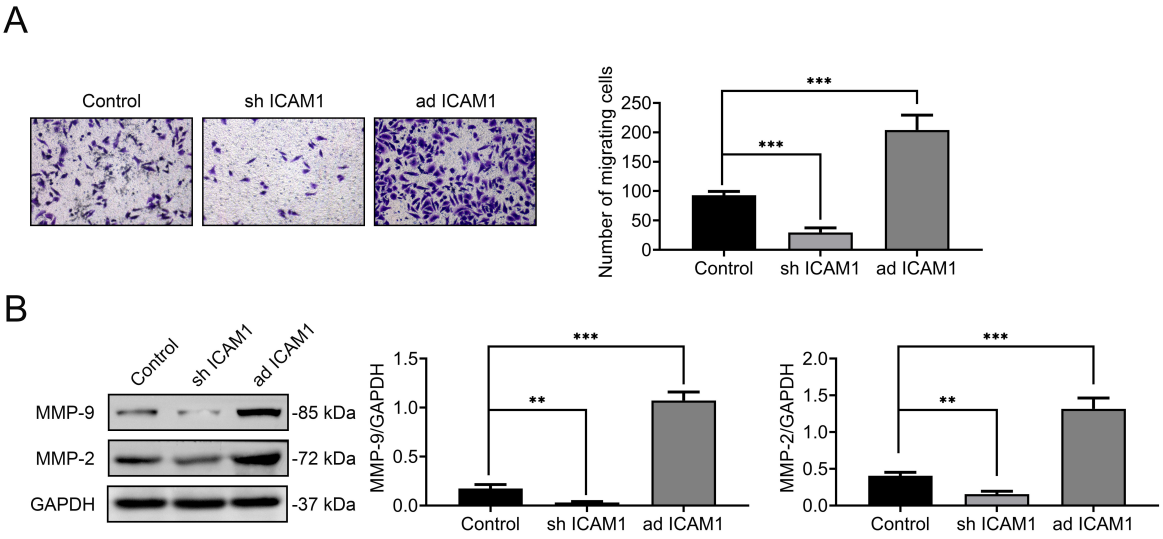

FIGURE 2. ICAM1 promotes the migration of DPSCs. (A) Transwell migration assay quantifying migrated DPSCs in control, ad ICAM1, and sh ICAM1 groups. (B) Western blot analysis of MMP-2 and MMP-9 expression levels in each group. Data are presented as mean ± SD; **$p < 0.01$, ***$p < 0.001$; technical replicates, n = 3. ICAM1: intercellular adhesion molecule 1; MMP: Matrix Metalloproteinase; GAPHD: Glyceraldehyde-3-Phosphate Dehydrogenase.

odontogenic lineage commitment and mineralization potential. Its increased expression further supports the role of ICAM1 in promoting odontoblast differentiation [18].

Alizarin Red S staining was used to assess mineralized matrix deposition following induction. The ad ICAM1 group exhibited the highest level of mineralization, while the sh ICAM1 group displayed the least matrix deposition (Fig. 3B,C). According to the results of the ALP activity measurement, after seven days of osteogenesis induction (ALP is an early marker of osteogenic differentiation), the ALP activity of ad ICAM1 was higher than that of the control group, whereas the ALP activity of sh ICAM1 was lower (Fig. 3D). Together, these results suggest that ICAM1 overexpression can enhance the osteogenic potential of DPSCs *in vitro*, whereas ICAM1 knockdown suppresses this capacity.

3.4 ICAM1 activates the TGF-β1/Smad signaling pathway

To investigate the molecular mechanism by which ICAM1 regulates DPSC differentiation, we examined the activation status of the TGF-β/Smad signaling pathway. Western blot analysis revealed that ICAM1 overexpression led to increased expression of TGF-β1, along with elevated phosphorylation levels of Smad2 and Smad3. In contrast, ICAM1 knockdown reduced the expression of TGF-β1 and the phosphorylation of Smad2 and Smad3 (Fig. 4). These findings suggest that ICAM1 activates the TGF-β/Smad signaling pathway in DPSCs.

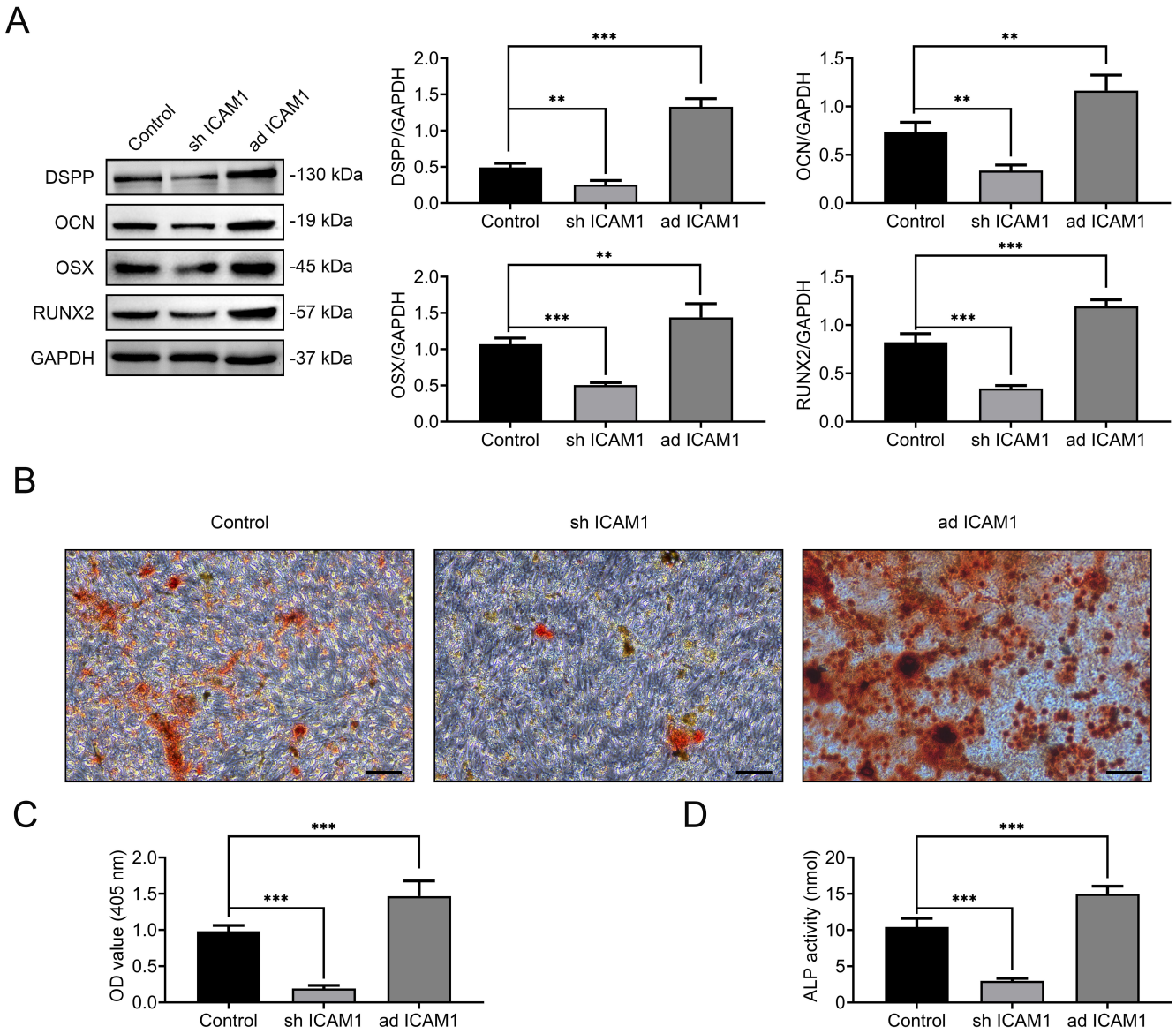

FIGURE 3. ICAM1 enhances the odontogenic differentiation potential of DPSCs. (A) Western blot analysis of odontogenic markers DSPP, OCN, RUNX2, and OSX in DPSCs under different ICAM1 expression conditions. (B,C) Alizarin Red S staining and quantitative analysis of mineralized matrix deposition. (D) ALP activity measurement after 7 days of osteogenic induction. Data are presented as mean ± SD; $^{**}p < 0.01$, $^{***}p < 0.001$; technical replicates, n = 3. Abbreviations: DSPP: dentin sialophosphoprotein; OCN: osteocalcin; RUNX2: runt-related transcription factor 2; OSX: osterix; ICAM1: intercellular adhesion molecule 1; GAPHD: Glyceraldehyde-3-Phosphate Dehydrogenase; OD: Optical Density; ALP: Alkaline Phosphatase.

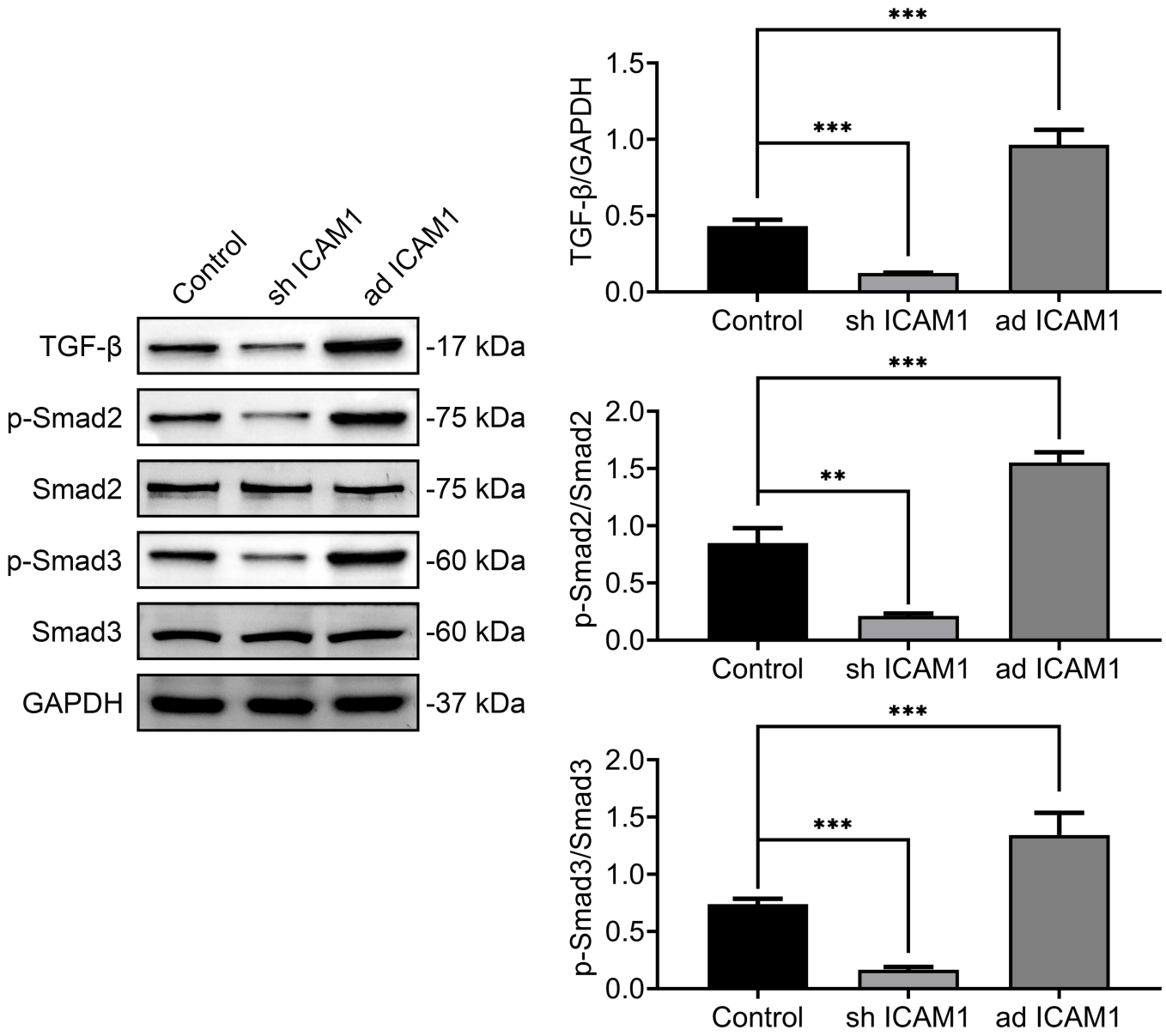

FIGURE 4. ICAM1 activates the TGF-β1/Smad signaling pathway. Western blot analysis showing expression levels of TGF-β1, total and phosphorylated Smad2 and Smad3 in DPSCs following ICAM1 modulation. Data are presented as mean ± SD; $^{**}p < 0.01$, $^{***}p < 0.001$; technical replicates, n = 3. ICAM1: intercellular adhesion molecule 1; TGF: transforming growth factor; Smad: Mothers Against Decapentaplegic Homolog; GAPHD: Glyceraldehyde-3-Phosphate Dehydrogenase.

3.5 Knockdown of TGF-β1 reverses the effect of ICAM1 on promoting odontoblast differentiation

To determine whether the pro-differentiation effects of ICAM1 are mediated via the TGF-β/Smad pathway, we silenced TGF-β1 in ICAM1-overexpressing DPSCs. Western blot analysis showed that TGF-β1 knockdown attenuated ICAM1-induced phosphorylation of Smad2 and Smad3, confirming suppression of pathway activation (Fig. 5A). Functionally, TGF-β1 knockdown reversed the ICAM1-mediated increase in DPSC viability as assessed by CCK-8 assay (Fig. 5B). Additionally, the expression levels of odontogenic markers including DSPP, OCN, RUNX2, and OSX were markedly reduced following TGF-β1 knockdown in ad ICAM1 cells (Fig. 5C). Consistently, Alizarin Red S staining and ALP activity assays demonstrated that mineralized matrix deposition and early osteogenic activity were also suppressed when TGF-β1 was silenced (Fig. 5D,E). Collectively, these findings indicate that the TGF-β/Smad signaling pathway is required for ICAM1-induced enhancement of odontoblast differentiation and DPSC viability, highlighting this axis as a critical mediator of ICAM1 function in odontogenic regulation.

4. Discussion

Stem cells are essential for tissue development and homeostasis due to their intrinsic capacity for self-renewal and multilineage differentiation. Similar to other organs, tooth development requires the coordinated involvement of various stem cell populations, regulated by specific signaling pathways [19]. Human DPSCs were first isolated by Shi, who

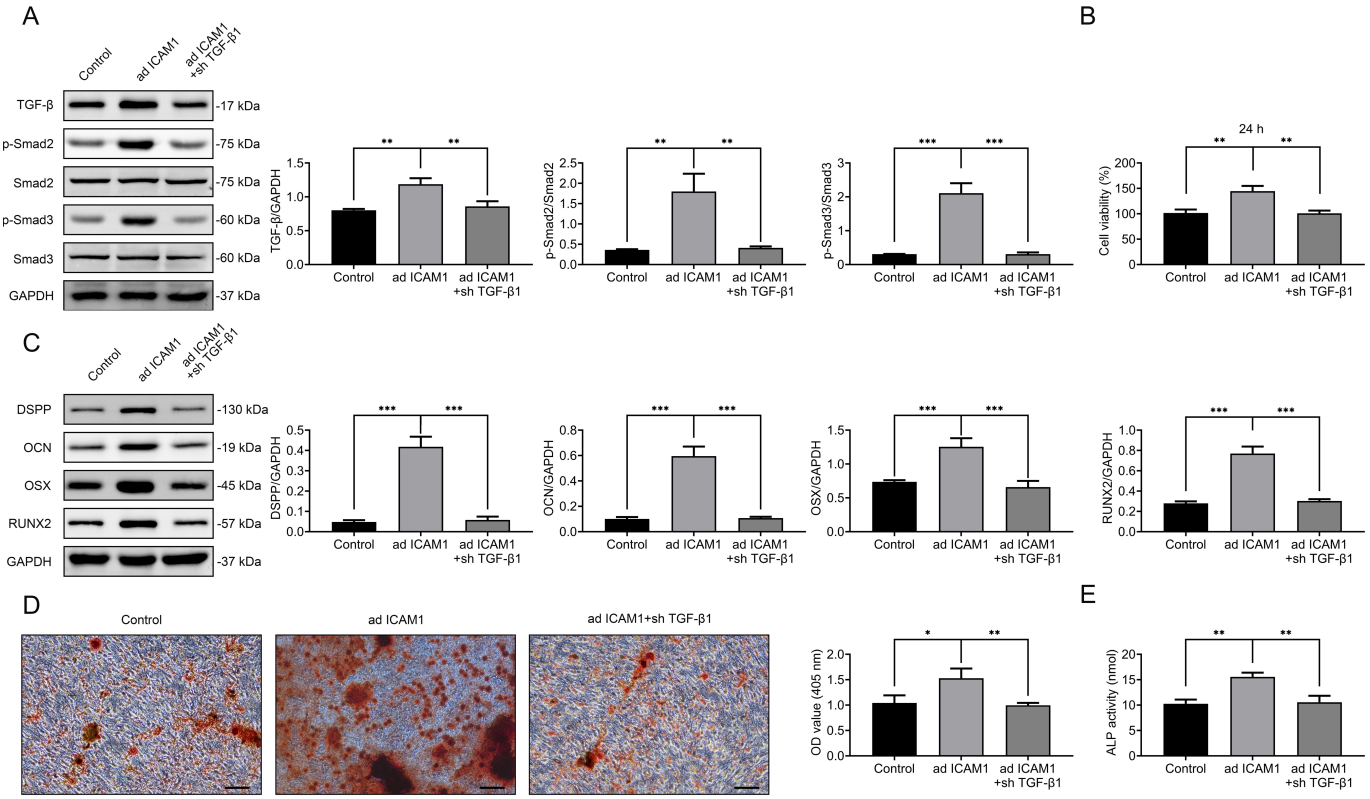

FIGURE 5. Knockdown of TGF-β1 reverses the ICAM1-induced enhancement of odontoblast differentiation. (A) Western blot analysis of TGF-β1, Smad2, p-Smad2, Smad3, and p-Smad3 following TGF-β1 knockdown in ad ICAM1-expressing DPSCs. (B) CCK-8 assay assessing cell viability. (C) Western blot analysis of DSPP, OCN, RUNX2, and OSX expression. (D) Alizarin Red S staining of mineralized matrix. (E) ALP activity assay. Data are presented as mean ± SD; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; technical replicates, n = 3. ICAM1: intercellular adhesion molecule 1; TGF: transforming growth factor; DSPP: dentin sialophosphoprotein; OCN: osteocalcin; RUNX2: runt-related transcription factor 2; OSX: osterix; Smad: Mothers Against Decapentaplegic Homolog; GAPHD: Glyceraldehyde-3-Phosphate Dehydrogenase; OD: Optical Density; ALP: Alkaline Phosphatase.

demonstrated their ability to self-renew, exhibit clonogenicity, proliferate rapidly, and differentiate into multiple lineages [20]. Odontoblasts, which are columnar and polarized cells located along the outermost region of the dental pulp, originate from DPSCs and are responsible for dentin synthesis. These cells also express DSPP, an important marker of odontoblastic differentiation [21]. However, precisely directing the differentiation of DPSCs into odontoblasts remains a major challenge in regenerative endodontics.

The molecule ICAM1 is a key surface molecule involved in cellular adhesion and migration. Recent evidence has shown that ICAM1 can influence mesenchymal stem cell behavior. For example, microRNA-221 targeting of ICAM1 has been reported to enhance endogenous stem cell migration and calvarial defect repair in rats [22], and melatonin has been shown to regulate ICAM1-mediated osteogenic differentiation in bone marrow-derived mesenchymal stem cells [12]. Furthermore, ICAM1 facilitates bone metastasis in triple-negative breast cancer by activating integrin-dependent TGF-β/Epithelial-Mesenchymal Transition (EMT) signaling [16]. In line with these findings, the present study demonstrates that ICAM1 promotes odontoblastic differentiation of DPSCs by activating the TGF-β/Smad signaling pathway. Conversely, silencing ICAM1 suppressed this differentiation, accompanied by downregulation of Smad2/3 phosphorylation.

Cell proliferation and migration are vital for tissue maintenance and regeneration [23]. In response to dentin injury, DPSCs proliferate and migrate toward the lesion, where they differentiate into odontoblast-like cells that secrete reparative dentin [24]. *In vitro*, DPSC differentiation into an odontoblast phenotype is characterized by the polarization of cell bodies and the formation of mineralized nodules [25]. DSPP is a definitive marker for odontoblast differentiation and plays an active role in their maturation [26]. In addition to DSPP, other late-stage mineralization markers, such as OCN, RUNX2 and OSX, are expressed during both odontoblast and osteoblast differentiation [27]. Our results demonstrate that ICAM1 overexpression enhances DPSC proliferation and migration, upregulates the expression of DSPP, RUNX2, OSX, and OCN, and promotes mineralized matrix deposition, thereby supporting its role in odontogenic differentiation.

TGF-β is a secreted cytokine that exerts paracrine effects on neighboring cells, initiating intracellular signaling cascades that regulate numerous biological functions, including proliferation, differentiation, and tissue repair [28]. Upon binding to its receptor complex, TGF-β1 activates the canonical Smad signaling pathway by inducing phosphorylation of Smad2 and Smad3. These phosphorylated Smads form a trimeric complex

with Smad4, which translocates into the nucleus to regulate the transcription of target genes [29]. Studies using transgenic mouse models have demonstrated that TGF-β signaling is essential for tooth development and odontoblastic differentiation. Additionally, recent research has shown that this pathway is involved in alveolar bone formation and root development [30, 31]. Herein, our findings support the role of ICAM1 in activating the TGF-β/Smad pathway. ICAM1 overexpression increased TGF-β1 levels and Smad2/3 phosphorylation, while TGF-β1 knockdown reversed these effects. Moreover, the enhanced proliferation, migration, and odontogenic differentiation of DPSCs induced by ICAM1 were also suppressed when TGF-β1 was silenced. These results suggest that ICAM1 promotes DPSC differentiation into odontoblasts by activating the TGF-β/Smad signaling pathway.

The study has several limitations. Firstly, it was conducted entirely *in vitro*, which may not fully reflect the complex *in vivo* environment of dental pulp tissue, necessitating further *in vivo* validation. Secondly, the use of viral transduction for overexpression and knockdown of ICAM1 and TGF-β1 could potentially introduce off-target effects or unintended cellular responses. Additionally, the limited number of DPSC lines used may not be representative of the broader population, affecting the reproducibility and generalizability of the results. Finally, the study assessed the effects of ICAM1 on DPSCs over a relatively short period (up to 21 days), and long-term functional outcomes remain unexplored.

In vitro, ICAM1 has been shown to stimulate DPSC migration and promote odontoblast differentiation, indicating its potential utility in dental pulp regeneration. However, the process of pulp regeneration is complex and involves not only the recruitment and differentiation of odontoblasts but also the repair of blood vessels and nerve tissues within the pulp. Additional *in vivo* studies are required to determine whether ICAM1 can mediate the homing of stem cells from periapical tissues or the circulatory system into the root canal space to facilitate complete pulp regeneration. Our findings demonstrate that ICAM1 significantly enhances the proliferation and odontogenic differentiation of DPSCs, suggesting its important role in regulating their biological behavior and providing a cellular foundation for regenerative applications. Since inflammatory responses are inevitable following dental pulp injury, moderate ICAM1 expression may contribute to maintaining immune balance and promoting tissue repair. Nonetheless, a major technical challenge that remains is how to efficiently and safely deliver ICAM1-targeting gene editing tools or pharmacological agents into the pulp tissue while minimizing potential damage to surrounding structures.

5. Conclusions

In summary, this study demonstrates that ICAM1 activates the TGF-β/Smad signaling pathway and enhances the odontogenic differentiation potential of human DPSCs. These findings underscore the role of ICAM1 in promoting odontoblast differentiation and provide new insights into potential strategies for improving vital pulp therapy.

AVAILABILITY OF DATA AND MATERIALS

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

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

JYL and JL—designed the study; completed the experiment and supervised the data collection; analyzed the data; interpreted the data. ZLZ—prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Xie Z, Shen Z, Zhan P, Yang J, Huang Q, Huang S, *et al*. Functional dental pulp regeneration: basic research and clinical translation. International Journal of Molecular Sciences. 2021; 22: 8991.

[2] Igna A, Mircioaga D, Boariu M, Stratul SI. A diagnostic insight of dental pulp testing methods in pediatric dentistry. Medicina. 2022; 58: 665.

[3] Goyal V. Pediatric endodontics. International Journal of Clinical Pediatric Dentistry. 2022; 15: S1–S2.

[4] Hassan R, Kouchaje C, Almattit H, Alnassar I. Evaluation of the onset of pulpal, soft tissue anesthesia, and pain using buffered lidocaine in children: a randomized-controlled trial. Signa Vitae. 2024; 20: 33–39.

[5] Elnawam H, Abdelmougod M, Mobarak A, Hussein M, Aboualmakarem H, Girgis M, *et al*. Regenerative endodontics and minimally invasive dentistry: intertwining paths crossing over into clinical translation. Frontiers in Bioengineering and Biotechnology. 2022; 10: 837639.

[6] Qiao X, Tang J, Dou L, Yang S, Sun Y, Mao H, *et al*. Dental pulp stem cell-derived exosomes regulate anti-inflammatory and osteogenesis in periodontal ligament stem cells and promote the repair of experimental periodontitis in rats. International Journal of Nanomedicine. 2023; 18: 4683–4703.

[7] Katata C, Sasaki JI, Li A, Abe GL, Nor JE, Hayashi M, *et al*. Fabrication of vascularized DPSC constructs for efficient pulp regeneration. Journal of Dental Research. 2021; 100: 1351–1358.

[8] Sui B, Wu D, Xiang L, Fu Y, Kou X, Shi S. Dental pulp stem cells: from discovery to clinical application. Journal of Endodontics. 2020; 46: S46–S55.

[9] Singh M, Thakur M, Mishra M, Yadav M, Vibhuti R, Menon AM, *et al*. Gene regulation of intracellular adhesion molecule-1 (ICAM-1): a

molecule with multiple functions. Immunology Letters. 2021; 240: 123–136.

[10] Bui TM, Wiesolek HL, Sumagin R. ICAM-1: a master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. Journal of Leukocyte Biology. 2020; 108: 787–799.

[11] Makino Y, Fujikawa K, Matsuki-Fukushima M, Inoue S, Nakamura M. Role of innate inflammation in the regulation of tissue remodeling during tooth eruption. Dentistry Journal. 2021; 9: 7.

[12] Li Y, Feng C, Gao M, Jin M, Liu T, Yuan Y, *et al*. MicroRNA-92b-5p modulates melatonin-mediated osteogenic differentiation of bone marrow mesenchymal stem cells by targeting ICAM-1. Journal of Cellular and Molecular Medicine. 2019; 23: 6140–6153.

[13] Liu YF, Zhang SY, Chen YY, Shi K, Zou B, Liu J, *et al*. ICAM-1 deficiency in the bone marrow niche impairs quiescence and repopulation of hematopoietic stem cells. Stem Cell Reports. 2018; 11: 258–273.

[14] Zhang Y, Zhang Y, Chen Y, Wu W, Sun J, Zhang X, *et al*. Impact of intercellular adhesion molecule 1-positive dental pulp stem cells in deep caries progression. International Endodontic Journal. 2025; 58: 1184–1196.

[15] Liu S, Yan X, Guo J, An H, Li X, Yang L, *et al*. Periodontal ligament-associated protein-1 knockout mice regulate the differentiation of osteoclasts and osteoblasts through TGF-beta1/Smad signaling pathway. Journal of Cellular Physiology. 2024; 239: e31062.

[16] Chen M, Wu C, Fu Z, Liu S. ICAM1 promotes bone metastasis via integrin-mediated TGF-beta/EMT signaling in triple-negative breast cancer. Cancer Science. 2022; 113: 3751–3765.

[17] Chen J, Xu H, Xia K, Cheng S, Zhang Q. Resolvin E1 accelerates pulp repair by regulating inflammation and stimulating dentin regeneration in dental pulp stem cells. Stem Cell Research & Therapy. 2021; 12: 75.

[18] Kim Y, Kang SW, Ye JR, Kim SE, Chae YK, Nam OH. Osteogenic potential of Frondoside A in human periodontal ligament cells: an RNA-Seq analysis. Journal of Periodontal & Implant Science. 2024; 54: 393–404.

[19] Li P, Ou Q, Shi S, Shao C. Immunomodulatory properties of mesenchymal stem cells/dental stem cells and their therapeutic applications. Cellular & Molecular Immunology. 2023; 20: 558–569.

[20] Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, *et al*. Stem cell properties of human dental pulp stem cells. Journal of Dental Research. 2002; 81: 531–535.

[21] Yuan X, Liu M, Cao X, Yang S. Ciliary IFT80 regulates dental pulp stem cells differentiation by FGF/FGFR1 and Hh/BMP2 signaling. International Journal of Biological Sciences. 2019; 15: 2087–2099.

[22] Chang W, Kim R, Park SI, Jung YJ, Ham O, Lee J, *et al*. Enhanced healing of rat calvarial bone defects with hypoxic conditioned medium from mesenchymal stem cells through increased endogenous stem cell migration via regulation of ICAM-1 targeted-microRNA-221. Molecular Cell. 2015; 38: 643–650.

[23] Tsutsui TW. Dental pulp stem cells: advances to applications. Stem Cells and Cloning. 2020; 13: 33–42.

[24] Yu L, Zeng L, Zhang Z, Zhu G, Xu Z, Xia J, *et al*. Cannabidiol rescues TNF-alpha-inhibited proliferation, migration, and osteogenic/odontogenic differentiation of dental pulp stem cells. Biomolecules. 2023; 13: 118.

[25] Lampiasi N. The migration and the fate of dental pulp stem cells. Biology. 2023; 12: 742.

[26] Kucukkaya Eren S, Bahador Zirh E, Zirh S, Sharafi P, Zeybek ND. Combined effects of bone morphogenetic protein-7 and mineral trioxide aggregate on the proliferation, migration, and differentiation of human dental pulp stem cells. Journal of Applied Oral Science. 2022; 30: e20220086.

[27] Liu Y, Liu N, Na J, Li C, Yue G, Fan Y, *et al*. Wnt/beta-catenin plays a dual function in calcium hydroxide induced proliferation, migration, osteogenic differentiation and mineralization *in vitro* human dental pulp stem cells. International Endodontic Journal. 2023; 56: 92–102.

[28] Heydarpour F, Sajadimajd S, Mirzarazi E, Haratipour P, Joshi T, Farzaei MH, *et al*. Involvement of TGF-beta and autophagy pathways in pathogenesis of diabetes: a comprehensive review on biological and pharmacological insights. Frontiers in Pharmacology. 2020; 11: 498758.

[29] Shi Y, Wang S, Zhang W, Zhu Y, Fan Z, Huang Y, *et al*. Bone marrow mesenchymal stem cells facilitate diabetic wound healing through the restoration of epidermal cell autophagy via the HIF-1α/TGF-β1/SMAD pathway. Stem Cell Research & Therapy. 2022; 13: 314.

[30] He XY, Sun K, Xu RS, Tan JL, Pi CX, Wan M, *et al*. Spatial signalling mediated by the transforming growth factor-beta signalling pathway during tooth formation. International Journal of Oral Science. 2016; 8: 199–204.

[31] Gao P, Liu C, Dong H, Li Q, Chen Y. TGF-beta promotes the proliferation and osteogenic differentiation of dental pulp stem cells a systematic review and meta-analysis. European Journal of Medical Research. 2023; 28: 261.

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