Regenerative Therapy 25 (2024) 77–84

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

CD73-positive pediatric urethral mesenchymal stem-like cell-derived small extracellular vesicles stimulate angiogenesis

Shilin Zhang*, Jierong Li, Chunjing Li, Xumin Xie, Jun He, Fengsheng Ling, Bowei Li, Huayan Wu, Zhilin Li, Jianwei Zhen, Guoqing Liu

Department of Urology, Foshan Maternity & Child Healthcare Hospital, Foshan 528000, China

ARTICLE INFO

Article history:
Received 15 November 2023
Received in revised form
24 November 2023
Accepted 7 December 2023

Keywords:
Hypospadias
Mesenchymal stem cells
Small extracellular vesicles
Angiogenesis
CD73

ABSTRACT

Introduction: Angiogenesis plays an important role in the repair of urethral injury, and stem cells and their secretomes can promote angiogenesis. We obtained pediatric urethral mesenchymal stem-like cells (PU-MSLCs) in an earlier study. This project studied the pro-angiogenic effect of PU-MSLC-derived small extracellular vesicles (PUMSLC-sEVs) and the underlying mechanisms.

Materials and methods: PUMSLCs and PUMSLC-sEVs were cultivated and identified. Then, biological methods such as the ethynyl deoxyuridine (EdU) incorporation assay, Cell Counting Kit-8 (CCK-8) assay, scratch wound assay, Transwell assay, and tube formation assay were used to study the effect of PUMSLC-sEVs on the proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVECs). We explored whether the proangiogenic effect of PUMSLC-sEVs is related to CD73 and whether adenosine (ADO, a CD73 metabolite) promoted angiogenesis. GraphPad Prism 8 software was used for data analysis.

Results: We observed that PUMSLC-sEVs significantly promoted the proliferation, migration, and tube-forming abilities of HUVECs. PUMSLC-sEVs delivered CD73 molecules to HUVECs to promote angiogenesis. The angiogenic ability of HUVECs was enhanced after treatment with extracellular ADO produced by CD73, and PUMSLC-sEVs further promoted angiogenesis by activating Adenosine Receptor A2A (A2AR).

Conclusions: These observations suggest that PUMSLC-sEVs promote angiogenesis, possibly through activation of the CD73/ADO/A2AR signaling axis.

1. Introduction

Hypospadias is the most common genital malformation in male children, with an incidence rate in newborn boys of 1/125-1/300 and increasing yearly [1]. Hypospadias not only hinders urination, sexual performance, and reproductive ability but also affects their psychological development [2], placing enormous pressure on their families. The only available treatment for hypospadias is surgery. More than 300 surgical methods for hypospadias have been reported in the literature, and the surgical methods have been continually improved. Postoperative complications such as urethral stricture and urinary fistula are still common [3], requiring reconstruction and repair, which indirectly reflects the difficulty treating this disease.

An important reason for the high incidence of complications of hypospadias reconstruction and repair is that the angiogenic capacity after injury is too weak to meet the needs of normal repair [4]. Mesenchymal stem cells (MSCs) and their secretomes can repair tissue and promote angiogenesis [5,6], but stem cells from different tissue sources differ in terms of accessibility, quantity, proliferation, immune regulation ability, cytokine profile secretion, and therapeutic potential for different diseases [7,8]. Our previous study [9] found that pediatric urethra mesenchymal stem-like cells (PU-MSLCs) may be an ideal repair material with easy accessibility and found that both PU-MSLCs and their culture supernatant can significantly promote the repair of urethral injury. Many biological

* Corresponding author. No.11, Renmin West Road, Chancheng District, Foshan, Guangdong, China.
E-mail address: zhang_40_1@163.com (S. Zhang).

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2023.12.002

properties of PU-MSLCs and the mechanisms involved in urethral reconstruction and repair with PU-MSLCs are still not clear.

Recent studies have found that MSCs can secrete small extracellular vesicles (sEVs) containing biologically active substances (such as proteins, lipids, mRNAs, and microRNAs), which transmit information between different cells and play a variety of biological roles. Mesenchymal stem cell–derived small extracellular vesicles (MSC-sEVs) perform well in tissue damage repair, immune regulation, and promoting angiogenesis [10,11]. We have found that umbilical cord mesenchymal stem cell exosomes (UCMSC-exo) can significantly promote the proliferation and migration of pediatric urethral smooth muscle cells (PUSMCs) in vitro by delivering CD73 molecules to PUSMCs and catalyzing the production of adenosine (ADO) and that the PI3K/AKT signaling pathway in PUSMCs is activated after ADO or UCMSC-exo intervention [12]. Based on this, we hypothesized that the effect of PU-MSLCs on urethral repair may be accomplished through the proangiogenic effect of PU-MSLC-derived small extracellular vesicles (PUMSLC-sEVs) and that CD73 molecules and ADO (a CD73 metabolite) promotes angiogenesis. This study analyzed the pro-angiogenic effect of PUMSLC-sEVs on vascular endothelial cells (VECs) and the underlying molecular mechanisms of its action, aiming to provide new theoretical support for urethral reconstruction and repair.

2. Methods and procedures

2.1. Isolation and identification of PUMSLCs

This experiment was approved by the Ethics Committee of Foshan Maternal and Child Health Hospital, and informed consent was obtained from the donors. According to the method previously established in our laboratory, pediatric urethral tissues were collected from children with hypospadias, minced, added to collagenase type I (Sigma, USA), digested at 37 °C for 4 h, filtered through a 70-mesh cell sieve (NEST, China), and then cultured with mesenchymal stem cell complete medium (Cyagen Biosciences, China). When the cells reached 80 % confluence, they were subcultured and purified according to the differential adhesion method.

Third-passage PUMSLCs were induced to differentiate in vitro by using adipogenesis (HUXXC-90031), osteogenesis (HUXXC-90021), and chondrogenesis (HUXXC-90041) induction differentiation kits (Cyagen, Guangzhou, China) to assess their adipogenic, osteogenic, and chondrogenic differentiation abilities. After 14–21 days of induction, the differentiated cells were stained with oil red O solution, alizarin red solution, and alcian blue solution, respectively.

The surface markers of the third-passage PUMSLCs were identified by flow cytometry. After PUMSLCs were incubated with fluorescently labeled CD29, CD34, CD45, CD73, CD90, CD105, and anti-HLA-DR monoclonal antibodies (mAbs) (Biolegend, USA) for 30 min, they were analyzed with a CytoFLEX flow cytometer (Beckman Coulter, USA).

2.2. Culture of human umbilical vein endothelial cells (HUVECs)

HUVECs were donated by the Department of Histology and Embryology, South China Agricultural University, Guangzhou, China. They were cultured in endothelial cell complete medium (ScienCell, USA) containing 10 % fetal bovine serum. After reaching 80 % confluence, the cells were propagated for 1:2 passage.

2.3. Extraction, purification, and identification of PUMSLC-sEVs

PUMSLCs at passages 3–8 were cultured in exosome-free medium for 48 h, and the cell supernatant was collected. The sEVs were isolated by ultracentrifugation and purified using an exosome purification and concentration kit (Exojuice, WeinaBio, China). Briefly, cells were removed by centrifugation at 300×*g* for 20 min, cell debris was removed by centrifugation at 10,000×*g* for 20 min, and sEVs were collected after centrifugation at 100,000×*g* for 70 min, followed by purification of exosomes according to Exojuice's instructions. The morphology of the exosomes was observed by transmission electron microscopy, the particle size of exosomes was analyzed by Flow NanoAnalyzer (NanoFCM, China), and the specific proteins associated with exosomes were detected by Western blotting (CD9, CD63, CD81 and TSG101).

2.4. Immunofluorescence staining

The extracted PUMSLC-sEVs were labeled with DiI dye, incubated with HUVECs at 37 °C for 3 h, fixed in 4 % paraformaldehyde for 15 min, and stained with FITC-phalloidin for the cytoskeleton for 45 min and DAPI for the nucleus. Fluorescence signals in cells were detected by fluorescence microscopy.

2.5. Ethynyl deoxyuridine (EdU) incorporation assay

HUVECs were seeded in six-well plates and cultured for 12 h, added to medium containing PUMSLC-sEVs, cultured for 12 h, treated with EdU solution (Beyotime, China) for 2 h, and incubated in the click reaction solution for 30 min at 37 °C in the dark. Cells were stained according to the kit instructions and then imaged under a fluorescence microscope, and the percentage of EdU-positive cells was calculated.

2.6. Cell counting kit-8 (CCK-8) assay

HUVECs were seeded in a 96-well plate at 5000 cells in 100 μl/well and cultured for 12 h. Then medium containing PUMSLC-sEVs was added, and they were cultured for 24 h. They finally received 10 μl of CCK-8 reagent and were incubated at 37 °C for 1.5 h. A microplate reader (Thermo MK3, USA) was used to measure the optical density (OD) at 450 nm.

2.7. Scratch wound assay

A culture insert (Ibidi, Martinsried, Germany) was placed in the middle of a 24-well culture plate. Then, 70 μl suspended HUVECs were seeded on the culture insert at 5×10^5 cells/ml. After 24 h, the culture insert was carefully removed, and PUMSLC-sEVs were added to treat HUVECs for 12 or 24 h. Cell migration was imaged under an inverted microscope (Olympus IX 71×, 100 × magnification, Olympus, Japan) and was calculated by ImageJ software v1.8 (National Institutes of Health, USA).

Table 1
Primers used for real-time PCR.

Accession number	Gene	Sequence (5′to3′)
NM_001025366.3	VEGF-F	ATCGAGTACATCTTCAAGCCAT
	VEGF-R	GTGAGGTTTGATCCGCATAATC
M37780.1	CD31-F	TCGTGGTCAACATAACAGAACT
	CD31-R	TTGAGTCTGTGACACAATCGTA
AF208487.1	HIF1A-F	AGTTCCGCAAGCCCTGAAAGC
	HIF1A-R	GCAGTGGTAGTGGTGGCATTAGC
BC065937.1	CD73-F	TGGGAGCTTACGATTTTGCACACC
	CD73-R	CGGATCTGCTGAACCTTGGTGAAG
NM_001101.5	β-actin-F	GGCATCCACGAAACTACATTCAATTCC
	β-actin-R	GTACCACCAGACAGCACTGTGTTG

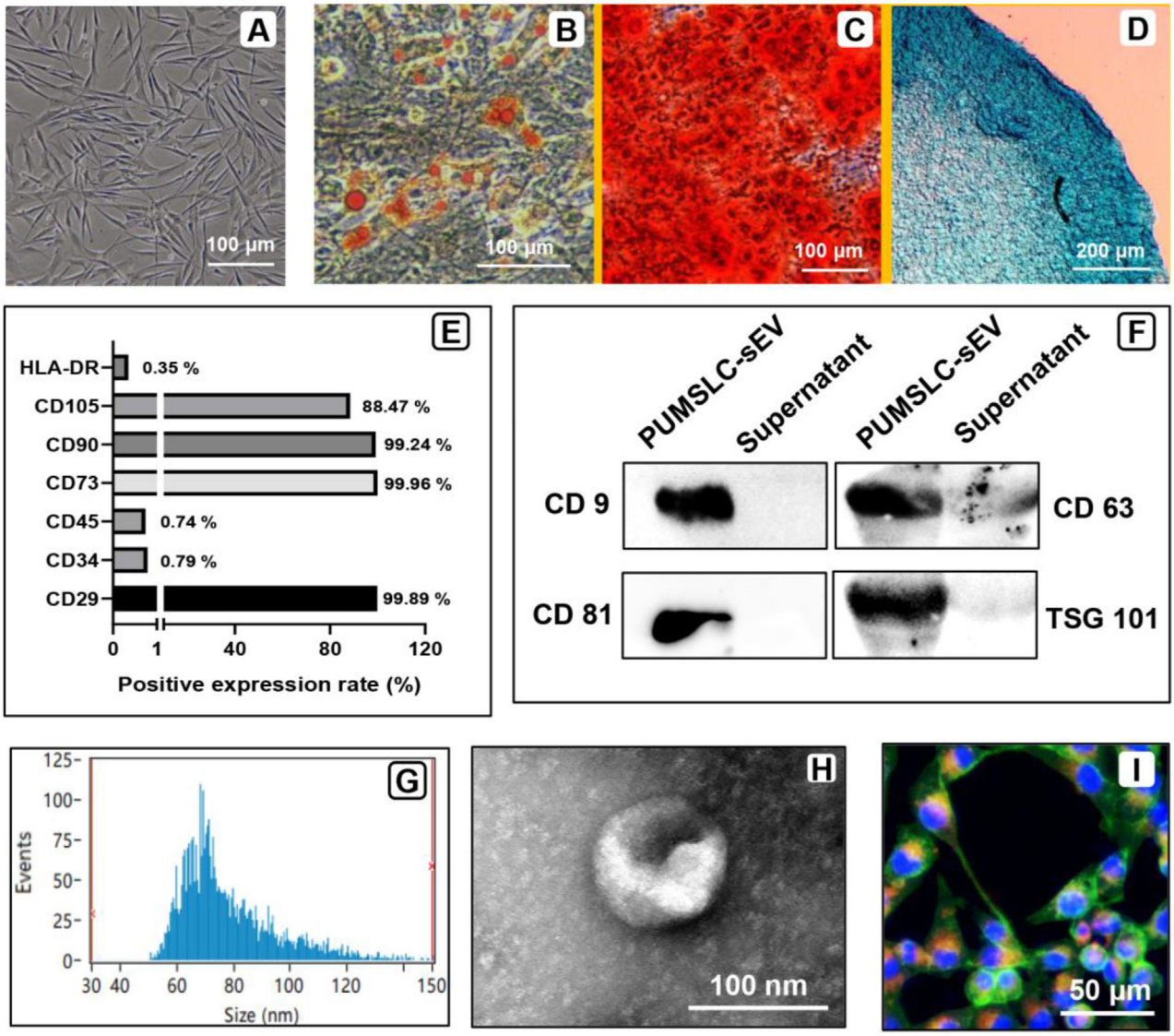

Fig. 1. Identification of PUMSLCs and PUMSLC-sEVs. (A) Morphological diagram of PUMSLCs: PUMSLCs differentiated into lipoblasts (B), osteoblasts (C), and chondroblasts (D) after in vitro induction. (E) Flow cytometry for MSC surface markers showed that PUMSLCs expressed high levels of CD29, CD73, CD90, and CD105 but did not express CD34, CD45, or HLA-DR. (F) Western blotting for the PUMSLC-sEV exosome marker proteins CD9, CD63, CD81, and TSG101. (G) NanoFCM determined the particle size of PUMSLC-sEVs. (H) Transmission electron microscopy showed that PUMSLC-sEVs were goblet-shaped. (I) PUMSLC-sEVs were endocytosed by HUVECs.

2.8. Transwell assay

HUVECs were resuspended in serum-free medium and seeded on the upper chamber of a Transwell system at 5×10^5 cells/ml (100 μl), and the culture medium containing PUMSLC-sEVs was added to the bottom chamber of the Transwell system. After 12 h or 24 h, the migrated cells (in the lower chamber) were counted after fixation and crystal violet staining.

2.9. Endothelial tube formation assay

Matrigel (Yeasen, China) was spread evenly on the bottom of a 96-well plate at 50 μl/well and placed in a 37 °C/5 % CO_2 incubator for 2 h to allow the Matrigel to solidify. HUVECs were seeded in 96-well plates at 30,000 cells/well, given medium containing PUMSLC-sEVs, and cultured for 4–8 h. Their tube formation was observed under an inverted microscope, and the lengths and branching points of the tubes formed were measured by ImageJ software.

2.10. qRT-PCR assay

The total RNA of exosomes was extracted and processed using TRIzol reagent (Thermo, USA), and then cDNA was synthesized by using the HiScript® III RT SuperMix for qPCR (+gDNA wiper) (Novizim, China). Quantitative PCR was performed using the ChamQ Universal SYBR qPCR Master Mix (Novizan, China) to detect the mRNA levels of CD73, HIF-1α, and VEGF. All gene sequences were downloaded from the GenBank, and primers were designed (Primer Premier 5) and synthesized (Sangon Biotech, China). The primer sequences are in Table 1.

2.11. Western blotting

The protein concentration was determined with a BCA kit, and 30 μg of total protein was resolved by 10 % SDS-PAGE and then transferred to PVDF membranes (Millipore, USA). Membranes were blocked with 5 % nonfat milk for 2 h and then incubated with

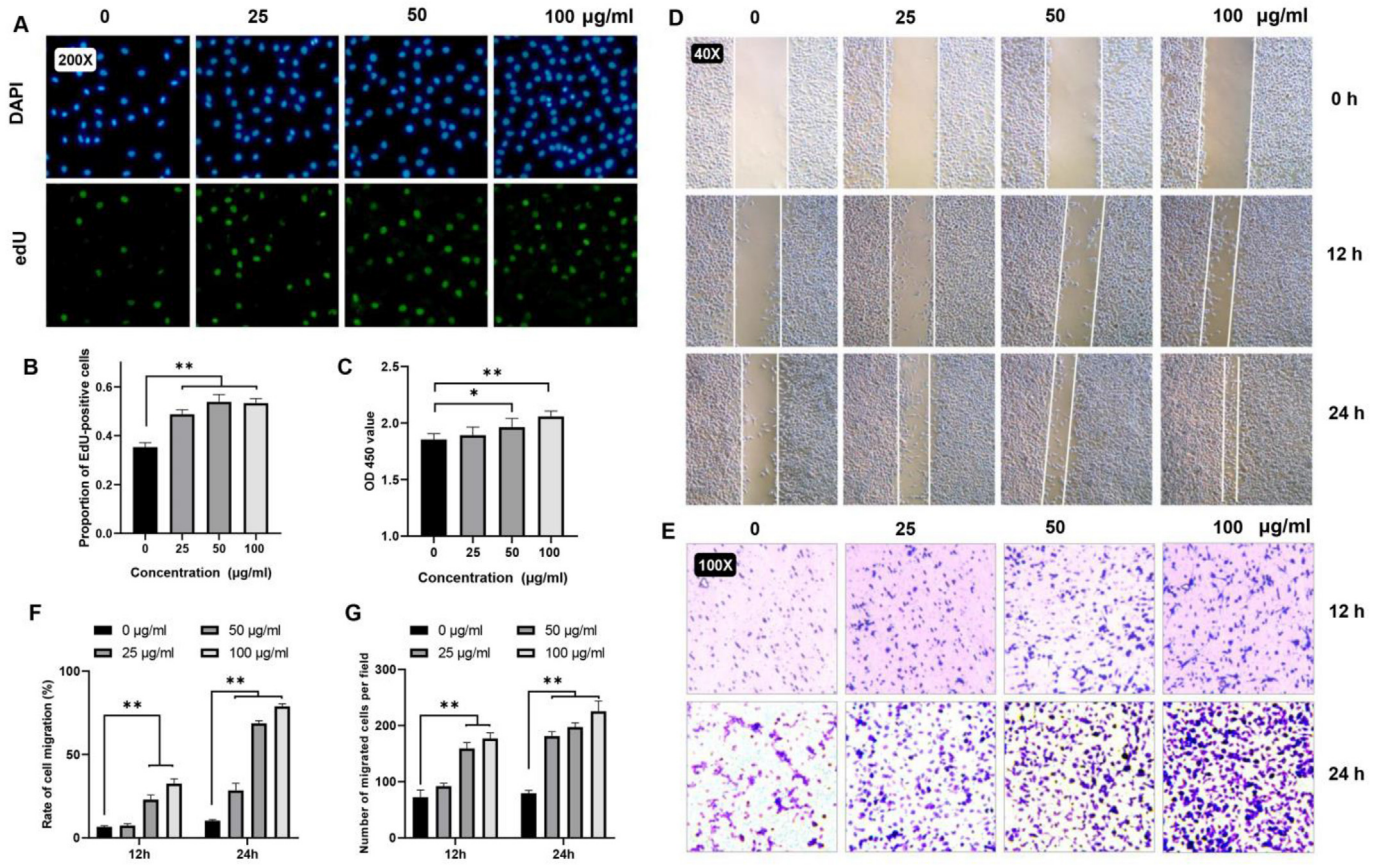

Fig. 2. PUMSLC-sEVs promote the proliferation and migration of HUVECs. (A and B) EdU incorporation assays showed that PUMSLC-sEVs promoted the proliferation of HUVECs. (C) CCK-8 assays showed that PUMSLC-sEVs promoted the proliferation of HUVECs. The scratch wound assay (D and F) and the Transwell assay (E and G) were consistent, both showing PUMSLC-sEVs promoted the migration of HUVECs.

primary antibodies (diluted at 1:1000) overnight at 4 °C. After washing three times with TBST, the membranes were added to the corresponding horseradish peroxidase–conjugated secondary antibodies (dilution ratio of 1:5000) and incubated for 1 h. After washing three times with TBST, a chemiluminescent reagent (Tanon, China) was dropped onto the membranes. Finally, the protein expression was observed with a chemiluminescence gel imaging analyzer (Tanon, China). The antibodies were anti-CD9 (AF5139, Affinity Biosciences, USA), anti-CD81 (DF2306, Affinity Biosciences, USA), anti-TSG101 (sc-7964, Santa Cruz Biotechnology, USA), anti-CD63 (sc-365604, Santa Cruz Biotechnology, USA), anti-CD73 (ab313339, Abcam, UK), and anti-β-actin (bs-0061R, Bioss Biotechnology, Bioss, China).

2.12. Statistical analysis

GraphPad Prism 8 software was used for data statistics. Measurement data are expressed as mean ± standard deviation (x ± s) and were assessed by Student's *t*-test. $P < 0.05$ indicated a significant difference, and $P < 0.01$ indicated an extremely significant difference.

3. Results

3.1. Identification of PUMSLCs and PUMSLC-sEVs

The isolated PUMSLCs were long, spindle-shaped adherent cells, showing a typical mesenchymal stem cell morphology (Fig. 1A). The PUMSLCs showed good adipogenic (Fig. 1B), osteogenic (Fig. 1C), and chondrogenic (Fig. 1D) differentiation abilities after induction in vitro. Flow cytometry showed that PUMSLCs expressed high levels of CD29, CD34, CD73, CD90, and CD105 but did not express CD45 or HLA-DR (Fig. 1E).

Western blotting showed that PUMSLC-sEVs expressed exosome marker proteins CD81, CD63, CD9, and TSG10 (Fig. 1F). Transmission electron microscopy showed that PUMSLC-sEVs had a typical disk-like structure (Fig. 1G). NanoFCM showed that the exosomes of PUMSLC-sEVs had diameters between 30 and 120 nm, mostly around 78.30 nm (Fig. 1H). Immunofluorescence showed that DiI-labeled exosomes were successfully endocytosed by HUVECs (Fig. 1I).

3.2. PUMSLC-sEVs promote proliferation, migration, and tube formation of HUVECs

The EdU assay (Fig. 2A and B) and CCK-8 assay (Fig. 2C) showed that PUMSLC-sEVs promoted the proliferation of HUVECs in a dose-dependent manner.

The migration results from the scratch wound assay (Fig. 2D, F) and the Transwell assay (Fig. 2E, G) were consistent, and PUMSLC-sEVs promoted the migration of HUVECs in a dose- and time-dependent manner. Low concentrations of PUMSLC-sEVs (25 μmol/L) had no effect on the migration of HUVECs.

The tube formation assay (Fig. 3A, B, C) showed that PUMSLC-sEVs promoted the tube formation of HUVECs in a dose-dependent manner. The qRT-PCR results (Fig. 3D and E) showed that PUMSLC-sEVs promoted the expression of the tube

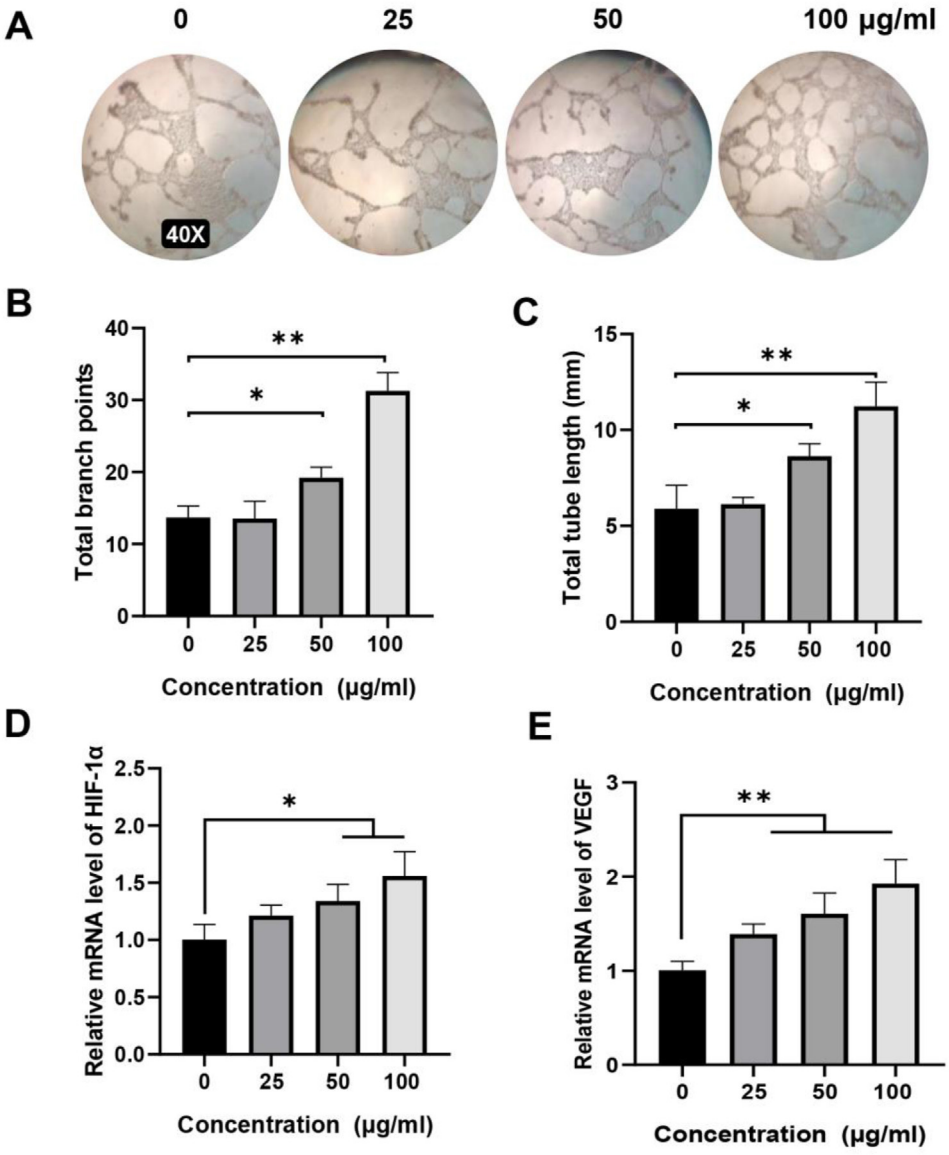

Fig. 3. PUMSLC-sEVs promote angiogenesis. (A) PUMSLC-sEVs significantly enhanced the tube-forming ability, as determined by the tube formation assay. (B and C) Quantitative analysis of the total length and number of tube branches. (D and E) PUMSLC-sEVs significantly enhanced the mRNA expression levels of HIF-1α and VEGF in HUVECs, as determined by qRT-PCR.

formation—related genes HIF-1α and VEGF and that low concentrations of PUMSLC-sEVs (25 μmol/L) had no effect on the angiogenesis of HUVECs.

3.3. PUMSLC-sEVs promote angiogenesis through CD73

qRT-PCR and Western blotting detected higher expression of CD73 mRNA (Fig. 4A) and protein (Fig. 4B) in PUMSLCs than in 293T cells (control group), and CD73 molecules was also highly expressed in PUMSLC-sEVs (Fig. 4B). To further explore whether the proangiogenic effect of PUMSLC-sEVs was related to CD73, we added a CD73 inhibitor (PSB12379) to the medium containing PUMSLC-sEVs. PSB12379 inhibited the activity of CD73 and thus reversed the proangiogenic effect of PUMSLC-sEVs (Fig. 4C, D, E).

3.4. ADO (a CD73 metabolite) promotes angiogenesis

Adding exogenous ADO to the culture medium of HUVECs significantly promoted the proliferation, migration, and tube formation of HUVECs (Fig. 5).

3.5. PUMSLC-sEVs mediate the effect of A2A ADO receptors on angiogenesis

qRT-PCR showed that the mRNA expression of A2A ADO receptors in HUVECs was upregulated after PUMSLC-sEV intervention (Fig. 6A). When we added an A2A ADO receptor antagonist (preladenant) to the medium containing PUMSLC-sEVs, the proangiogenic effect of PUMSLC-sEVs was inhibited (Fig. 6B, C, D).

4. Discussion

This work investigated whether PUMSLC-sEVs affect the proliferation, migration, and tube-forming abilities of HUVECs and conducted a preliminary exploration of the underlying molecular mechanisms. The results show that PUMSLC-sEVs can significantly promote the proliferation, migration, and tube formation of HUVECs and that this process may be related to their activation of the CD73/ADO/A2AR signaling axis.

MSCsEVs carry different cargo molecules (such as proteins, nucleic acids, and lipids) and have various biological effects, making

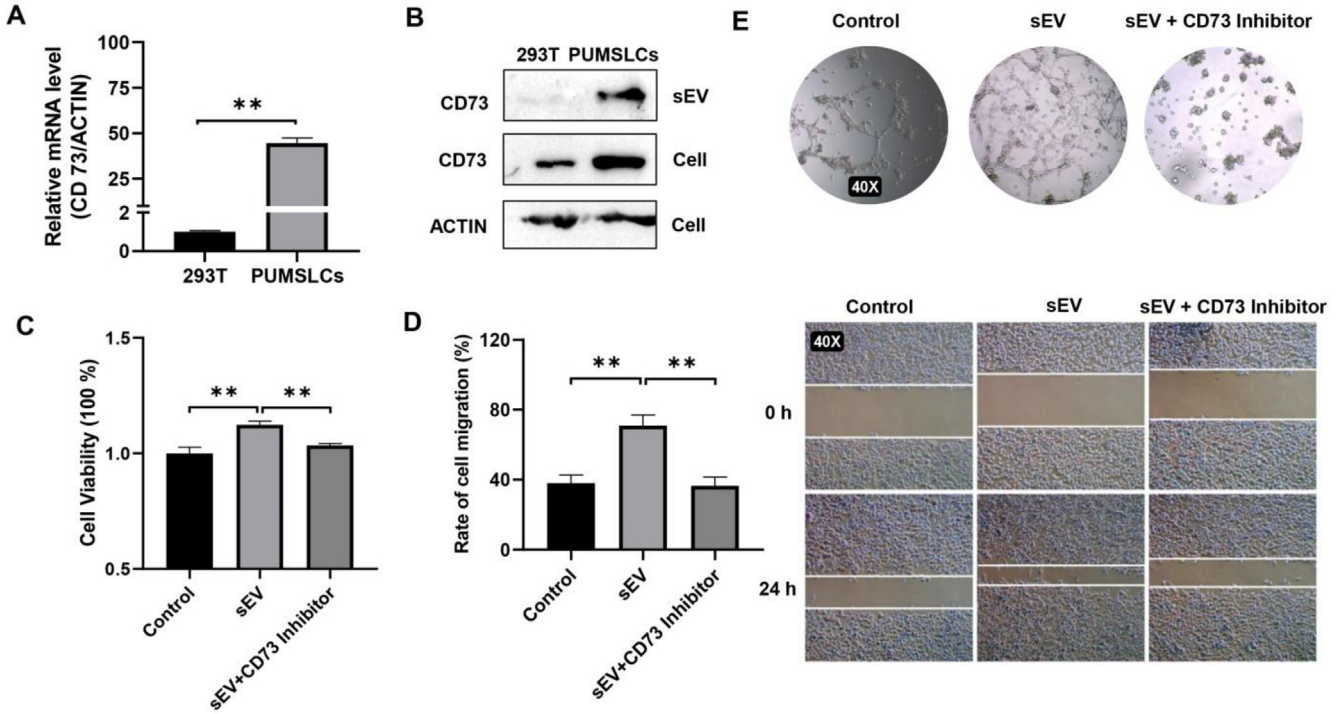

Fig. 4. The proangiogenic effect of CD73⁻positive PUMSLC-sEVs is reversed by CD73 inhibition. (A) qRT-PCR detection of CD73 in PUMSLCs (293T cells as a control group). (B) Western blotting detection of CD73 in PUMSLCs and PUMSLC-sEVs (293T cells as a control group). CCK-8 assay (C), scratch wound assay (D), and tube formation assay (E) showed that the inhibitor PSB12379 reversed the promoting effect of CD73-positive PUMSLC-sEVs on the proliferation, migration, and tube formation of HUVECs.

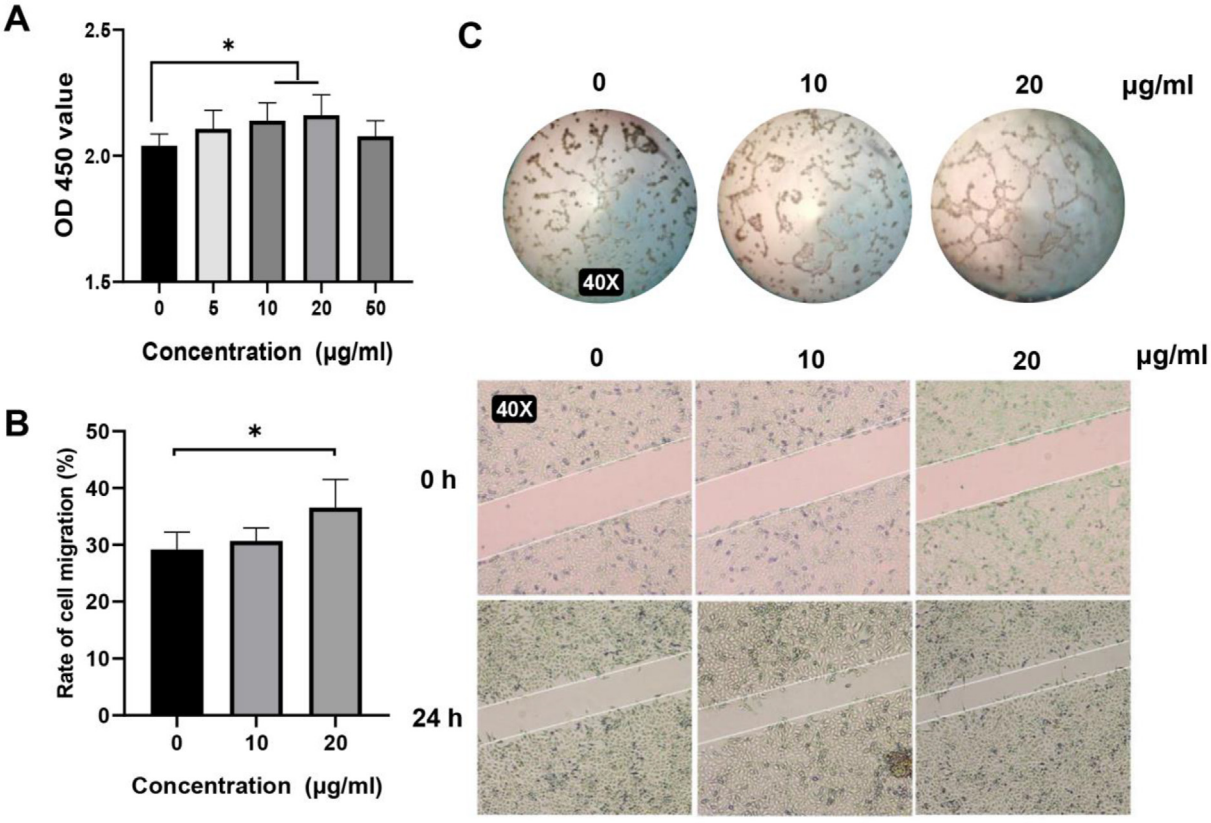

Fig. 5. Adenosine promotes the proliferation, migration, and tube formation of HUVECs. (A) CCK-8 assay and Transwell assay. (B) Tube formation assay. (C) Addition of exogenous adenosine significantly promoted the proliferation, migration, and tube formation of HUVECs in vitro.

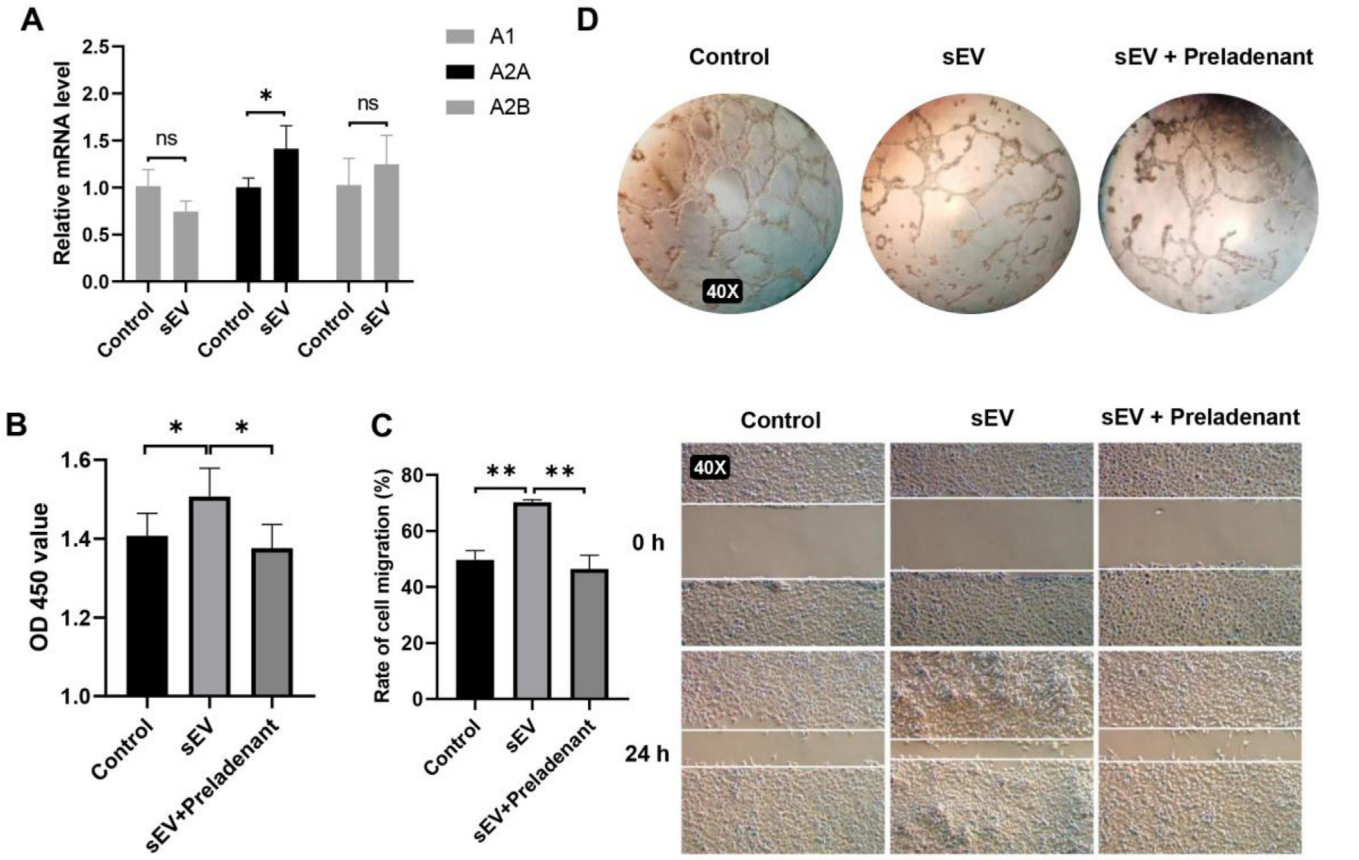

Fig. 6. CD73-positive PUMSLC-sEVs activate A2A adenosine receptors to promote angiogenesis. The qRT-PCR (A) showed that PUMSLC-sEVs activated the A2A adenosine receptors. CCK-8 assay (B), scratch wound assay (C), and tube formation assay (D) showed that preladenant (an adenosine receptor A2A antagonist) reversed the promoting effect of CD73-positive PUMSLC-sEVs on the proliferation, migration, and tube formation of HUVECs.

them an ideal therapeutic tool or various diseases [13,14]. Cell-free MSC-sEVs have broad clinical application prospects since they are more stable and easier to preserve, manage, and control, advantages that have been widely reported in various preclinical animal models and human clinical trials [15,16]. In this study, we first isolated PUMSLC-sEVs by ultracentrifugation and then characterized PUMSLC-sEVs by transmission electron microscopy, NanoFCM, and detection of the expression of exosome marker molecules (such as CD9, CD63, CD81, and TSG101).

Urethral repair is a complex process involving the proliferation and regeneration of epithelial cells, smooth muscle cells, and endothelial cells, all of which rely on blood vessels to deliver nutrients and oxygen [17,18]. Angiogenesis is an important mechanism for urethral repair, and VECs are the main cells maintaining the physiological functions of blood vessels [19]. MSC-sEVs can reduce tissue damage and play an important role in the regulation of VECs [20]. The process of angiogenesis mainly includes the degradation of the original vascular basement membrane to release endothelial cells; the proliferation, migration, and infiltration of VECs in the direction of stimulation; the formation of the vascular lumens; and the trimming and remodeling of vascular structures [21,22]. The proliferation, migration, and tube formation of VECs play a crucial role in angiogenesis [23]. MSCs-sEVs can markedly increase the levels of VEGF, TGF-β, and angiogenin secreted by VECs, thereby promoting angiogenesis [24]. sEVs function in two known ways: one is binding to cell membrane receptors of recipient cells through receptor-dependent regulation to directly activate the recipient cells, and the other is to encapsulate exosomes into cells through endocytosis and then release the proteins, mRNAs, miRNAs, etc., from the exosomes into the recipient cells [25,26]. MSC-sEVs are involved in angiogenesis, but whether MSC-sEVs can promote the proliferation, migration, and tube formation of VECs in the repair of urethral injury is still unclear. In this study, the proliferation, migration, and tube-forming abilities of HUVECs were significantly enhanced in the presence of PUMSLC-sEVs. These results show that PUMSLC-sEVs indeed promote angiogenesis, which is consistent with the finding of Motohiro Komaki that human placental MSC-derived exosomes play an important role in promoting angiogenesis [27].

This study found that PUMSLC-sEVs carried many CD73 molecules and that after the CD73 inhibitor PSB12379 was added to inhibit the CD73 activity in PUMSLC-sEVs, the proangiogenic effect of PUMSLC-sEVs disappeared. Furthermore, adding exogenous ADO (a CD73 metabolite) to the culture medium of HUVECs significantly promoted the angiogenesis of HUVECs. We infer that PUMSLC-sEVs deliver CD73 molecules to HUVECs, and this CD73 induces ADO production to promote angiogenesis. CD73 is overexpressed in tumor tissues and can promote tumor cell proliferation [28–30]. During tumor development, CD73 promotes endothelial cells to form new blood vessels [31].

In the experiments to determine which ADO receptor mediates the proangiogenic effect of PUMSLC-sEVs, we found that PUMSLC-sEVs promoted the activation of the A2A receptors. After we added A2A ADO receptor antagonist to the medium containing PUMSLC-sEVs, the proangiogenic effect of PUMSLC-sEVs was blocked.

5. Conclusion

Under these experimental conditions, PUMSLC-sEVs significantly promoted the proliferation, migration, and tube-forming abilities of HUVECs. We speculate that PUMSLC-sEVs promote angiogenesis by activating the CD73/ADO/A2AR signaling axis.

Data availability statement

The original contribution presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

All procedures in the present study were approved by the Ethics Committee of Foshan Maternity & Child Healthcare Hospital. Written informed consent was obtained from all donors.

Author contributions

SLZ: Experiment, Writing- Original draft preparation, and manuscript revision. JRL, CJL, XMX, and JH: Experiment, Original draft preparation. FSL and BWL: Data curation and Writing-Reviewing. HYW and ZLL: Supervision and language polishment. JWZ and GQL: Conceptualization and manuscript revision.

Funding

This study was supported by the Innovation Project of Women and Children medical research center affiliated to Foshan Institute of Fetal Medicine (FEYJZX-2023-007) and Guangdong Medical Science and Technology Research Fund Project (A2021240).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Thanks to the team of Deja Lab, Foshan for their help in the isolation and identification of primary cells and exosomes.

References

[1] Bouty A, Ayers KL, Pask A, Heloury Y, Sinclair AH. The genetic and environmental factors underlying hypospadias. Sex Dev 2015;9(5):239–59.
[2] Kiss A, Sulya B, Szasz AM, Romics I, Kelemen Z, Toth J, et al. Long-term psychological and sexual outcomes of severe penile hypospadias repair. J Sex Med 2011;8(5):1529–39.
[3] Mouravas V, Filippopoulos A, Sfoungaris D. Urethral plate grafting improves the results of tubularized incised plate urethroplasty in primary hypospadias. J Pediatr Urol 2014;10(3):463–8.
[4] Wang DJ, Li MY, Huang WT, Lu MH, Hu C, Li K, et al. Repair of urethral defects with polylactid acid fibrous membrane seeded with adipose-derived stem cells in a rabbit model. Connect Tissue Res 2015;56(6):434–9.
[5] Zou J, Yang W, Cui W, Li C, Ma C, Ji X, et al. Therapeutic potential and mechanisms of mesenchymal stem cell-derived exosomes as bioactive materials in tendon-bone healing. J Nanobiotechnol 2023;21(1):14.
[6] Qi X, Zhang J, Yuan H, Xu Z, Li Q, Niu X, et al. Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells repair critical-sized bone defects through enhanced angiogenesis and osteogenesis in osteoporotic rats. Int J Biol Sci 2016;12(7):836–49.
[7] Laplane L, Solary E. Towards a classification of stem cells. Elife 2019;8.
[8] Preynat-Seauve O, Krause KH. Stem cell sources for regenerative medicine: the immunological point of view. Semin Immunopathol 2011;33(6):519–24.
[9] Zhang S, Li J, Li C, Xuminxie, He J, Ling F, et al. Isolation and identification of a mesenchymal stem/stromal cell-like population from pediatric urethral tissue. In Vitro Cell Dev Biol Anim 2022;58(6):503–11.
[10] Liu L, Guo S, Shi W, Liu Q, Huo F, Wu Y, et al. Bone marrow mesenchymal stem cell-derived small extracellular vesicles promote periodontal regeneration. Tissue Eng 2021;27(13–14):962–76.
[11] Lu Y, Wang L, Zhang M, Chen Z. Mesenchymal stem cell-derived small extracellular vesicles: a novel approach for kidney disease treatment. Int J Nanomed 2022;17:3603–18.
[12] Zhang S, Li J, Li C, Xie X, He J, Ling F, et al. CD73-Positive small extracellular vesicles derived from umbilical cord mesenchymal stem cells promote the proliferation and migration of pediatric urethral smooth muscle cells through adenosine pathway. Front Bioeng Biotechnol 2022;10:895998.
[13] Xiao X, Xu M, Yu H, Wang L, Li X, Rak J, et al. Mesenchymal stem cell-derived small extracellular vesicles mitigate oxidative stress-induced senescence in endothelial cells via regulation of miR-146a/Src. Signal Transduct Targeted Ther 2021;6(1):354.
[14] Pathipati P, Lecuyer M, Faustino J, Strivelli J, Phinney DG, Vexler ZS. Mesenchymal stem cell (MSC)-Derived extracellular vesicles protect from neonatal stroke by interacting with microglial cells. Neurotherapeutics 2021;18(3):1939–52.
[15] Kou M, Huang L, Yang J, Chiang Z, Chen S, Liu J, et al. Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: a next generation therapeutic tool? Cell Death Dis 2022;13(7):580.
[16] Tang Y, Zhou Y, Li HJ. Advances in mesenchymal stem cell exosomes: a review. Stem Cell Res Ther 2021;12(1):71.
[17] Chan YY, Bury MI, Fuller NJ, Nolan BG, Gerbie EY, Hofer MD, et al. Effects of anti-inflammatory nanofibers on urethral healing. Macromol Biosci 2021;21(5):e2000410.
[18] Liang Y, Yang C, Ye F, Cheng Z, Li W, Hu Y, et al. Repair of the urethral mucosa defect model using adipose-derived stem cell sheets and monitoring the fate of indocyanine green-labeled sheets by near infrared-II. ACS Biomater Sci Eng 2022;8(11):4909–20.
[19] Wang B, Lv X, Li Z, Zhang M, Yao J, Sheng N, et al. Urethra-inspired biomimetic scaffold: a therapeutic strategy to promote angiogenesis for urethral regeneration in a rabbit model. Acta Biomater 2020;102:247–58.
[20] Zhang Y, Xie Y, Hao Z, Zhou P, Wang P, Fang S, et al. Umbilical mesenchymal stem cell-derived exosome-encapsulated hydrogels accelerate bone repair by enhancing angiogenesis. ACS Appl Mater Interfaces 2021;13(16):18472–87.
[21] Ma Q, Reiter RJ, Chen Y. Role of melatonin in controlling angiogenesis under physiological and pathological conditions. Angiogenesis 2020;23(2):91–104.
[22] Liu B, Zhou H, Zhang T, Gao X, Tao B, Xing H, et al. Loss of endothelial glucocorticoid receptor promotes angiogenesis via upregulation of Wnt/beta-catenin pathway. Angiogenesis 2021;24(3):631–45.
[23] Wang D, Wang J, Zhou J, Zheng X. The role of adenosine receptor A2A in the regulation of macrophage exosomes and vascular endothelial cells during bone healing. J Inflamm Res 2021;14:4001–17.
[24] Xia Y, Ling X, Hu G, Zhu Q, Zhang J, Li Q, et al. Small extracellular vesicles secreted by human iPSC-derived MSC enhance angiogenesis through inhibiting STAT3-dependent autophagy in ischemic stroke. Stem Cell Res Ther 2020;11(1):313.
[25] Liu X, Shao Y, Tu J, Sun J, Dong B, Wang Z, et al. TMAO-activated hepatocyte-derived exosomes impair angiogenesis via repressing CXCR4. Front Cell Dev Biol 2021;9:804049.
[26] Bardi GT, Al-Rayan N, Richie JL, Yaddanapudi K, Hood JL. Detection of inflammation-related melanoma small extracellular vesicle (sEV) mRNA content using primary melanocyte sEVs as a reference. Int J Mol Sci 2019;20(5).
[27] Komaki M, Numata Y, Morioka C, Honda I, Tooi M, Yokoyama N, et al. Exosomes of human placenta-derived mesenchymal stem cells stimulate angiogenesis. Stem Cell Res Ther 2017;8(1):219.
[28] Ghalamfarsa G, Kazemi MH, Raoofi MS, Masjedi A, Hojjat-Farsangi M, Azizi G, et al. CD73 as a potential opportunity for cancer immunotherapy. Expert Opin Ther Targets 2019;23(2):127–42.
[29] Chen YH, Lu HI, Lo CM, Li SH. CD73 promotes tumor progression in patients with esophageal squamous cell carcinoma. Cancers 2021;13(16).
[30] Munoz-Lopez S, Sanchez-Melgar A, Martin M, Albasanz JL. Resveratrol enhances A(1) and hinders A(2A) adenosine receptors signaling in both HeLa and SH-SY5Y cells: potential mechanism of its antitumoral action. Front Endocrinol 2022;13:1007801.
[31] Wang L, Tang S, Wang Y, Xu S, Yu J, Zhi X, et al. Ecto-5'-nucleotidase (CD73) promotes tumor angiogenesis. Clin Exp Metastasis 2013;30(5):671–80.