Enhancer of zeste homolog 2 enhances the migration and chemotaxis of dental mesenchymal stem cells

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Abstract
Objective: To investigate the function of enhancer of zeste homolog 2 (EZH2) in the migration and chemotaxis of human dental tissue-derived mesenchymal stem cells.
Methods: The expression of EZH2, C-X-C motif chemokine ligand 11 (CXCL11), CXCL16, and CXCR1 in stem cells from the apical papilla (SCAPs) was determined by real-time reverse transcription PCR and western blotting. The effects of EZH2 on the homing of SCAPs and the effects of EZH2-overexpressing SCAP culture supernatant on periodontal ligament stem cells (PDLSCs) were tested by scratch migration assays and transwell chemotaxis assays.
Results: EZH2 overexpression significantly enhanced the migration and chemotaxis of SCAPs and upregulated the expression of CXCL11, CXCL16, and CXCR1 in SCAPs. EZH2 depletion had the opposite effect, impairing the migration and chemotaxis of SCAPs and downregulating the expression of CXCL11, CXCL16, and CXCR1. The culture supernatant of EZH2-overexpressing SCAPs advanced the migration and chemotaxis of PDLSCs.
Conclusions: EZH2 evidently promoted the migration and chemotaxis of SCAPs by upregulating the expression of CXCL11, CXCL16, and CXCR1. Moreover, EZH2-overexpressing SCAPs enhanced the homing, migration, and chemotaxis of PDLSCs via paracrine signaling.

Keywords
Chemotaxis, enhancer of zeste homolog 2, human dental tissue-derived mesenchymal stem cells, migration, C-X-C motif chemokine, homing

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Introduction
Mesenchymal stem cells (MSCs) are pluripotent progenitor cells that can differentiate into a wide diversity of somatic cells such as chondrocytes, osteoblasts, myoblasts, adipocytes, and neural cells. They are also conducive to the repair and regeneration of corresponding tissues including cartilage, bone, muscle, fat, and tendons.1,2 By interaction with various biomolecules like chemokines, cytokines, and growth factors, MSCs contribute to the regeneration and repair of inflamed or damaged tissues through stem cell homeostasis and immunomodulation.3–6

Tissue engineering approaches have been explored for a range of oral and dental tissues.7 For this purpose, human dental tissue-derived mesenchymal stem cells (hDT-MSCs) of various kinds including stem cells from the apical papilla (SCAPs), dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), dental follicle stem cells (DFSCs), and stem cells from human exfoliated deciduous teeth have been used.8–13 SCAPs derive from a wide range of sources and are easy to obtain. They also have a higher proliferation rate and telomerase activity, as well as enhanced tissue regeneration and migration capacities compared with DPSCs.14–16 Their application is further expanded because of their low immunogenicity.17 The apical papilla of teeth is a developing organ and an effective source of cells for hard tissue regeneration such as root formation,18 while PDLSCs have the potential for periodontal regeneration through differentiating into fibroblasts, cementoblasts, and osteoblasts.19

In the context of stem cell-based therapies, the delivery of cells to the target site of inflammation, injury, or repair (homing) is very challenging. MSC homing is a complex process mediated by chemotactic cytokines, chemokine receptors, and endothelial co-receptors. It is also affected by culture conditions such as cell confluency, oxygen concentration, and passage numbers.20–23 However, it is not clear how to enhance the cell homing of hDT-MSCs. Nevertheless, because this mainly relies on cell migration, the chemotaxis ability is critical in determining the efficacy of MSC-based therapies.24 Therefore, improving the homing ability can be vital in optimizing regenerative medicine outcomes. MSCs can also accelerate tissue repair or regeneration through paracrine functions.25 enhancer of zeste homolog 2 (EZH2), a catalytic component of the polycomb repressive complex 2, acts as a methyltransferase for H3 lysine 27 trimethylation, modulates chromatin structure and gene expression, and recruits DNA methyltransferases for gene silencing.26,27 Recently, several studies reported that EZH2 has an important role in tumor cell metastasis. Additionally, EZH2 was found to promote tumor cell homing,28–30 while tumor suppressor genes such as p16/CDKN2A, BAP1, ARID1A, and DAB2IP were associated with EZH2 in multiple tumors.31–34 EZH2 also plays an essential role in maintaining the self-renewal and proliferative capabilities of stem cells,35 while EZH2 downregulation suppressed proliferation and promoted regeneration of human dental pulp cells.36 These findings suggest that EZH2 regulates dental pulp proliferation and regeneration. Indeed, its control of human dental pulp inflammation was reported in vitro with its ability to affect the transcription of proinflammatory cytokines interleukin (IL)-6, IL-8, and C-C motif chemokine ligand (CCL)2 by binding their promoters.37 The release of proinflammatory factors from dental pulp cells is likely to promote stem cell homing to manage pulp inflammation.38 It was previously shown that the depletion of EZH2 depressed BMSC migration by changing nuclear properties,39 but the role and
mechanism of EZH2 in the homing of hDT-MSCs remains unknown.

In preliminary findings, we used microarray analysis to show that the expression of 15 chemokines, including CXCL11, CXCL16, and CXCR1, was decreased after EZH2 knockdown in SCAPs. Therefore, the present study aimed to investigate the function and mechanism of EZH2 in the migration and chemotaxis of hDT-MSCs to explore its potential role in the homing of hDT-MSCs.

Materials and methods

Cell cultures

The present study was approved by the Institutional Research Ethics Committee, and all experiments involving human stem cells were conducted following guidelines set by Beijing Stomatological Hospital Capital Medical University (Beijing Stomatological Hospital Ethics Review No. 2011-02). Three human impacted third molar teeth were obtained from three healthy patients (aged 16–20 years) attending the Department of Oral and Maxillofacial Surgery (Beijing Stomatological Hospital) after obtaining informed consent. All teeth were flushed using sterile phosphate-buffered saline (PBS) and stored in this at 4°C. SCAPs and PDLSCs were then isolated, cultivated, and identified as previously described.15,40 Briefly, apical papillae were gently separated from the root by eye scissors, and PDLSCs were separated from the periodontal ligament in the middle third of the root. Apical papillae and periodontal ligament tissues were then immersed in a solution containing 4 mg/mL dispase (Roche Diagnostics Corp, Indianapolis, IN) and 3 mg/mL collagenase type I (Worthington Biochemical Corp, Lakewood, NJ) for 1 hour at 37°C. Single-cell suspensions were obtained using a 70-μm strainer (Falcon, BD Labware, Franklin Lakes, NJ). Stem cells were cultured in alpha Dulbecco’s modified Eagle’s medium containing 15% fetal bovine serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L glutamine (all Invitrogen, Carlsbad, CA) in a humidified incubator with 5% CO2 at 37°C. The medium was changed every 72 hours.

Plasmid construction and viral infection

Plasmids were constructed using standard techniques and were verified by restriction enzyme digests and sequencing.41 The EZH2-specific short hairpin RNAs (shRNA) was subcloned into the LV3 lentiviral vector with green fluorescent protein (GenePharma, Suzhou, China). Lentivirus packaging was performed by GenePharma. The human full-length EZH2 gene sequence was combined with an HA-tag using the gene synthesis method,42 and cloned into the pQCXIN retroviral vector at the BamHI and AgeI restriction sites (Clontech Laboratories, Mountain View, CA, USA). Retroviral packaging was performed according to the manufacturer’s protocol (Clontech Laboratories). Approximately 2 × 10⁶ SCAPs were plated in 10-mm dishes and transfected for 12 hours using lentiviruses or retroviruses mixed with 6 μg/mL polybrene (Sigma Aldrich, St. Louis, MO). After 48 hours, infected SCAPs were screened with suitable antibiotics: those infected with EZH2sh or Consh lentiviruses were screened with 1 μg/mL puromycin (Invitrogen) for 7 days, while those infected with HA-EZH2 or vector retrovirus were selected with 400 μg/mL G418 (Invitrogen) for 10 days. Scrambled shRNA (Scramsh) supplied by GenePharma was used as a control. The target sequence of EZH2 shRNA (EZH2sh) was: 5'-GCTGATGAAGGAGTTAGG-3'.

Real-time reverse transcription (RT)-PCR

Total RNA was extracted from infected SCAPs using TRIzol reagent (Invitrogen).
According to the manufacturer’s protocol, target cDNA was amplified from 2 µg RNA using oligo (dT) or random hexamers and reverse transcriptase. Finally, real-time PCR reactions were executed by the iCycler iQ Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA) and the SYBR Green PCR kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Gene expression changes were calculated by the delta CT method. Online D-LUX Designer™ software (Invitrogen) was used to design the following primers (Supplementary Table 1).

**Western blot analysis**

Infected SCAPs were dissolved in radio immunoprecipitation assay buffer containing 1% Nonidet P 40, 1 mM ethylenediaminetetraacetic acid, 1% sodium dodecyl sulphate (SDS), 10 mmol/L Tris-HCl, 50 mmol/L β-glycerophosphate, 50 mmol sodium fluoride, and 1: 100 proteinase inhibitor cocktail. Samples were separated on 15% SDS polyacrylamide gels and transferred to polyvinylidene difluoride membranes using semi-dry transfer apparatus (BioRad, Hercules, CA) at 2.5 A, 2.5 V for 7 minutes. The membranes were then immersed in 5% dehydrated milk for 1 hour and incubated with primary antibodies overnight at 4°C, then with an anti-mouse/rabbit IgG secondary antibody conjugated with horseradish peroxidase for 40 minutes at room temperature (Promega, Madison, WI). Immune complexes were detected using SuperSignal reagents (Pierce, Rockford, IL). Primary antibodies included a monoclonal antibody against β-actin (Applygen Technologies, Beijing, China), monoclonal antibody against EZH2 (Abcam, Cambridge, MA), monoclonal antibody against CXCL11 (Zenbio, Chengdu, China), monoclonal antibody against CXCL16 (Bioss, Beijing, China), and a monoclonal antibody against CXCR1 (Bioss).

**Scratch migration assays**

The scratch migration assay was conducted to assess cell migration. Infected SCAPs (∼2 × 10^5 cells/well) were seeded onto six-well plates (n = 3) and proliferated to achieve 95% confluency. Cells were then washed twice using PBS, and cultivated for 1 hour at 37°C in minimum essential medium α (α-MEM) without serum. Three scratches were made per well with a 1000-µL pipette tip, and each well was washed three times using PBS. Cells were then grown in fresh α-MEM culture medium without serum. Images from the same viewpoint were collected by an inverted microscope (Olympus, Tokyo, Japan) at 0, 24, and 48 hours after the scratch. Image-Pro1.49v software (National Institutes of Health, Bethesda, MD) was used to calculate the void area (VA), height, and relative width (Area% = VA/height) in each group.

SCAPs infected with vector or EZH2 were cultured for 72 hours prior to the collection of culture medium (CM) supernatant. PDLSCs (∼2 × 10^5 cells/well) were seeded onto six-well plates and expanded to 95% confluency. After scratching as described above, PDLSCs were cultured in α-MEM culture medium containing 50% Vector-CM or EZH2-CM. The migration distance of cells was observed and measured at 0, 24, and 48 hours post-scratch using an inverted microscope and Image-Pro1.49v software as described above.

**Transwell chemotaxis assays**

Transwell chemotaxis assays were conducted on SCAPs or PDLSCs (∼4.0 × 10^4) cultured in 100 µL α-MEM culture media without serum in transwell chambers with 8-µm pore size membranes (Corning, Costar, MA). The lower chamber of the 24-well plate contained 600 µL of α-MEM culture media or α-MEM culture media containing 50% Vector-CM or EZH2-CM containing 15% fetal bovine
serum (Invitrogen). Cells were cultured at 37°C for 24 hours, washed twice with PBS, fixed with anhydrous formaldehyde at 4°C for 20 minutes, and dipped in 1% crystal violet for 3 minutes at room temperature. All chemotaxis assays were conducted in triplicate and evaluated simultaneously. Ultimately, we calculated the number of transferred cells in random fields of microscopy (Olympus) at 200× magnification.

Statistical analysis

Data and statistical calculations were handled using SPSS 17.0 software (SPSS Inc., Chicago, IL). The Student’s t-test was used to assess the significance between the average of two groups with \( P \leq 0.05 \) deemed significant.

Results

Overexpression of EZH2 promoted migration and chemotaxis in SCAPs

To investigate the role of EZH2 in SCAP chemotaxis and migration, we infected SCAPs with a retroviral vector carrying the HA-EZH2 sequence. Western blotting and real-time RT-PCR showed that EZH2 was significantly overexpressed in HA-EZH2-infected SCAPs compared with the vector-only group (Figure 1A, B; \( P < 0.01 \)). Additionally, the scratch migration assay revealed that EZH2 overexpression significantly increased the migration capacity of SCAPs at 24 and 48 hours compared with the control group (Figure 1C, D; \( P < 0.01 \)). Moreover, the transwell chemotaxis assay showed that overexpression of EZH2 significantly enhanced the chemotaxic capability of SCAPs at 24 hours (Figure 1E, F; \( P < 0.05 \)).

Depletion of EZH2 depressed migration and chemotaxis of SCAPs

To study the role of EZH2 in the migration and chemotaxis of SCAPs, we knocked down its expression using lentiviral EZH2 shRNA. Real-time RT-PCR and western blotting detected significantly reduced EZH2 expression following lentiviral delivery (Figure 2A, B; \( P < 0.05 \)). Scratch migration assay data showed that enhanced depletion of EZH2 significantly repressed the migration capability of SCAPs at 24 hours compared with the control group (Figure 2C, D; \( P < 0.05 \)). Moreover, the transwell chemotaxis assay revealed that EZH2 depletion significantly reduced SCAP chemotaxis at 24 hours (Figure 2E, F; \( P < 0.05 \)).

Conditioned medium of HA-EZH2-overexpressing SCAPs promoted migration and chemotaxis in PDLSCs

To clarify the paracrine function of EZH2, we next incubated PDLSCs in culture supernatants from SCAPs carrying the vector alone or HA-EZH2, then assessed them using scratch migration and transwell chemotaxis assays. CM from EZH2-overexpressing SCAPs significantly increased the migration capacity of PDLSCs at 48 hours compared with the control group (Figure 3A, B; \( P < 0.05 \)). Moreover, the transwell assay revealed that the culture supernatant of EZH2-overexpressing SCAPs significantly enhanced chemotaxis in PDLSCs at 24 hours compared with the control group (Figure 3C, D; \( P < 0.01 \)).

EZH2 up-regulated the expression of CXCL11, CXCL16, and CXCR1 in SCAPs

To determine the function of EZH2 in regulating SCAP chemokines, we used real-time RT-PCR and western blotting to evaluate their expression. These revealed significantly enhanced CXCL11, CXCL16, and CXCR1 expression in EZH2-overexpressing SCAPs (Figure 4A, B; \( P < 0.05 \)). Consistent with this, the depletion of EZH2 significantly downregulated
Figure 1. Overexpression of EZH2 promoted the migration and chemotaxis ability of SCAPs. The HA-EZH2 sequence was inserted into a retroviral vector and then transduced into SCAPs via retroviral
the expression of CXCL11, CXCL16, and CXCR1 in SCAPs (Figure 4C, D; \( P < 0.05 \)).

To verify that SCAPs infected with vectors carrying EZH2 maintain their stem cell properties, we next inserted the HA-EZH2 sequence into a retroviral vector and transduced this into SCAPs. We then determined the expression of stem cell genes including *NANOG*, *SOX2*, and *OCT4* by real-time RT-PCR and found no significant change after overexpressing EZH2 (Supplementary Figure 1A).

We used real-time RT-PCR to investigate changes in the expression of tumor suppressor genes *P16/CDKN2A*, *BAP1*, *DAB2IP*, and *ARID1A* in SCAPs infected with EZH2 vectors. We observed no significant change in the expression of *BAP1*, *DAB2IP*, and *ARID1A* following EZH2 overexpression but detected a significant increase in the expression of *P16/CDKN2A* (Supplementary Figure 1B; \( P < 0.05 \)).

**Discussion**

In the present study, we found that EZH2 significantly upregulated the expression of CXCL11, CXCL16, and CXCR1 in SCAPs and significantly enhanced cell migration and chemotaxis. EZH2 was previously detected by immunohistochemistry and immunofluorescence analyses in dental pulp cells during inflammation, regeneration, and proliferation, suggesting its association with various physiological and pathological phases. For instance, EZH2 was reduced in infected pulp tissues, while EZH2 inhibition affected the inflammatory expression of interleukins and alkaline phosphatase activity.\(^3^6\)

We identified no obvious change in the expression of *NANOG*, *SOX2*, or *OCT4* following EZH2 overexpression in SCAPs, suggesting that the stem cell properties of SCAPs were still maintained. We also found no difference in the expression of *BAP1*, *DAB2IP*, or *ARID1A*, but detected a significant increase in the expression of *P16/CDKN2A* after overexpressing EZH2 in SCAPs. Previous research showed that EZH2-dependent repression of p16/CDKN2A led to the cell cycle progression observed in multiple cancers.\(^3^1\) Therefore, the role of EZH2 in regulating proto-oncogenes and tumor suppressor genes in stem cells needs further study.

Consistent with our current results, EZH2 downregulation has been shown to suppress BMSC migration.\(^3^9\) However, its role appears to vary remarkably among human tissues. For instance, overexpression of EZH2 in prostate cells is associated with the risk of developing benign hyperplasia in the prostate and prostatitis,\(^4^3\) while EZH2 activation in pancreatic islet beta cells and dental pulp regeneration preserves the pluripotency of associated stem cells and promotes their proliferative activity and differentiation.\(^4^4\) Additionally, EZH2 inhibition may potentiate osteogenic and myogenic regeneration and enhance the expression of differentiation genes,\(^4^5\) providing support for its use in stem cell-based therapy. Moreover, EZH2 has been identified as a key regulator of tumor angiogenesis through its role in stimulating

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**Figure 1. Continued**

infection. (A, B): Real-time RT-PCR and western blotting indicated that EZH2 was overexpressed in SCAPs transduced with the HA-EZH2 retrovirus compared with vector-only cells. GAPDH and \( \beta \)-actin were used as internal controls. (C, D): The scratch-simulated wound migration assay showed that EZH2 overexpression promoted the migration of SCAPs at 24 and 48 hours (images were taken from the same field of view). Scale bar: 100 \( \mu \)m. (E, F): The transwell chemotaxis assay showed that EZH2 overexpression promoted the chemotaxis of SCAPs at 24 hours. Scale bar: 50 \( \mu \)m. Error bars represent SD (\( n = 3 \)). **\( P < 0.01 \); Student's t test.
Figure 2. Depletion of EZH2 depressed the migration and chemotaxis ability of SCAPs. EZH2 was knocked down in SCAPs using lentiviral EZH2 shRNA. (A, B): Real-time RT-PCR and western blotting indicated that EZH2 was depressed in SCAPs following transduction of shRNA. (C, D): The scratch-simulated wound migration assay showed that EZH2 knock-down reduced the migration of SCAPs at 24 hours (images were taken from the same field of view). Scale bar: 100 µm. (E, F): The transwell chemotaxis assay showed that EZH2 knock-down reduced the chemotaxis of SCAPs at 24 hours. Scale bar: 50 µm. Error bars represent SD (n = 3). *P < 0.05; Student’s t test.
vascular endothelial growth factor (VEGF); conversely, the suppression of EZH2 from tumor endothelial cells reduced angiogenesis by upregulating angiogenesis inhibitors.\(^{46}\) EZH2 has also been associated with fibrogenesis,\(^ {47}\) with EZH2 inhibition shown to diminish myofibroblast activity in an animal model.\(^ {48}\)

Several studies\(^ {49-51}\) investigated the enhancement of stem cell homing, either by modulation with chemokine receptors or adjusting target tissues to release corresponding chemokines. Chemokines are an important family of signaling molecules comprising four subfamilies, CXC, CC, C, and CX3C. They are known for their ability...
to trigger leukocyte activation in inflammatory processes as well as their critical roles in MSC chemotaxis and migration, with overexpression of CXCR4 shown to enhance MSC homing to the bone marrow of NOD/SCID mice. Additionally, CCR1 overexpression significantly enhanced chemokine-induced mouse MSC migration.
while BMSCs are thought to migrate under the action of CCL25.55 CX3CL1 was previously shown to activate Jak2/Stat5a/ERK1/2 through CX3CR1 receptors to complete the directional migration of BMSCs to the brain in a therapeutic role.56 Moreover, CCL5 enhanced the migration of dermal stromal cells and adipose stem cells in an in vitro wound healing assay.57

Genetic modification has been used to improve the homing capability of MSCs by promoting their innate properties.58 For example, overexpression of the α4 subunit of the VLA-4 integrin increased the homing of MSCs to bone marrow in a mouse model.59 The mitogen-activated protein kinase (MAPK) signaling pathway, including ERK1/2, Jun amino-terminal kinases 1/2/3, and p38-MAPK, regulates cell homing through SDF-1/CXCR4.60 Moreover, the phosphoinositide 3-kinase/Akt signaling pathway also functions in cell homing in association with the RhoA/ROCK signaling pathway,61 while the Notch signaling pathway regulates CXCR4 expression and MSC migration.62

A variety of chemokines including CXCL2, CXCL10, and CCL16 are expressed in healthy dental pulp and are thought to enhance the migration of immune cells in the local environment.63 These findings are in agreement with our observations that EZH2 regulates SCAP homing by controlling the expression of CXCL11, CXCL16, and CXCR1. CXCL11 has a latent capacity in tumor intervention through its functional specificity.64 It is a target gene of microRNA-206, which was reported to inhibit the migration and invasion of prostate cancer cells by down-regulating CXCL11.65 Additionally, chemokines CXCL10 and CXCL11 were shown to recruit human MSCs by chemotaxis assays,66 while CXCL16 appears to advance the proliferation and invasion of lung cancer by altering the nuclear factor κB pathway.67 Furthermore, CCL11, CXCL16, CXCL12, and CXCL13 were found to enhance the bidirectional migration of MSCs across bone marrow endothelial cells.68 CXCR1 as a G protein-coupled receptor and IL-8 receptor alpha can be bonded and activated by CXCL6 and CXCL8,69 and overexpression of the IL-8 receptor gene and CXCR1 were shown to improve the capability of human umbilical cord blood-derived MSCs to migrate to gliomas.70 This evidence indicates that CXCL11, CXCL16, and CXCR1 may contribute to the homing of MSCs, but the regulatory molecular mechanism was unclear.

hDT-MSCs are currently under study as a superior cell source for multipotent stem cells in tissue repair.10 They secrete paracrine factors that play significant roles in the repair and regeneration of tissues,71 and their migration can be potentiated using biomaterials that advance paracrine effects.72 Similarly, pretreatment of MSCs by pro-inflammatory cytokines enhanced the metastasis and migration of cholangiocarcinoma cells via paracrine function.73 Moreover, stem cell-mediated paracrine signaling altered the proliferation and migration of native vocal fold fibroblasts in vitro.74

In the present study, the culture supernatant of EZH2-overexpressing SCAPs enhanced the migration and chemotaxis ability of PDLSCs, indicating the likelihood of a paracrine mechanism. Additionally, EZH2 upregulation increased the expression of CXCL11, CXCL16, and CXCR1 in SCAPs. This indicates that EZH2 has the potential to be used in therapeutic approaches for the regeneration of dental pulp and periodontal tissues. For instance, the ability to potentiate fibrogenesis and angiogenesis through the EZH2–VEGF paracrine circuit may aid pulpal revascularization or the regeneration of periodontal ligaments which both require fibroblastic activity.
Several obstacles remain to be overcome before hDT-MSCs can be widely used, including understanding cell homing, directed differentiation, cell proliferation, and modulation mechanisms. Although several studies have reported hDT-MSC homing to target tissues, which is crucial for stem cell therapy, this requires further research.\textsuperscript{50} It also remains to be determined how EZH2 regulates the homing and paracrine functions of MSCs. A limitation of our current study is that we only investigated specific dental stem cells (SCAPs and PDLSCs). Given that EZH2 interactions vary widely among tissues and organs, further research is needed to understand the response of various stem cells and cell lines at the molecular level. Additionally, the present study did not explore the association of EZH2 with various differentiated cell lines such as fibroblast, odontoblasts, and osteoblasts in the context of dental tissue regeneration.

In conclusion, our study revealed that EZH2-overexpressing SCAPs showed enhanced migration and chemotaxis capabilities, and increased expression of CXCL11, CXCL16, and CXCR1. Additionally, EZH2 promoted migration and chemotaxis in PDLSCs via a paracrine mechanism. These findings highlight the possibility of promoting hDT-MSC-based cell therapies and regenerative applications including dental pulp and periodontal ligament regeneration.

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Authors’ contributions
HM performed cell culture studies, participated in data analysis and interpretation, and drafted the manuscript. SD and FY performed molecular biology studies, and participated in data analysis and interpretation. HY, CY, and LG participated in molecular biology studies and statistical analysis. ZS was responsible for the study conception and design, data analysis and interpretation, manuscript writing, and final approval of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declaration of conflicting interests
The authors declare that there is no conflict of interest.

Ethics approval and consent to participate
Tooth tissues were acquired on the basis of guidelines established by the Beijing Stomatological Hospital, Capital Medical University (Ethical Committee Agreement by Beijing Stomatological Hospital Ethics Review No. 2011–02) with informed patient consent.

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References
1. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143–147.
2. Barry FP and Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. Int J Biochem Cell Biol 2003; 36: 568–584.
3. Melzer C, Yang Y and Hass R. Interaction of MSC with tumor cells. Cell Commun Signal 2016; 14: 20.
4. Mathieu N, Frick J, Thierry D, et al. Human mesenchymal stem cells home specifically to radiation-injured tissues in a nonobese diabetes/severe combined immunodeficiency mouse model. Br J Radiol 2007; 80: S49–S55.

5. Oh W, Kim DS, Yang YS, et al. Immunological properties of umbilical cord blood-derived mesenchymal stromal cells. Cell Immunol 2008; 251: 116–123.

6. Rumman M and Dhawan J. Concise review: quiescence in adult stem cells: biological significance and relevance to tissue regeneration. Stem Cells 2015; 33: 2903–2912.

7. Zafar MS, Khurshid Z and Almas K. Oral tissue engineering progress and challenges. Tissue Eng Regen Med 2015; 12: 387–397.

8. Yang B, Qiu Y, Zhou N, et al. Application of stem cells in oral disease therapy: progresses and perspectives. Front Physiol 2017; 8: 197.

9. Liu J, Yu F, Sun Y, et al. Concise reviews: characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells 2015; 33: 627–638.

10. Na S, Zhang H, Huang F, et al. Regeneration of dental pulp/dentine complex with a three-dimensional and scaffold-free stem-cell sheet-derived pellet. J Tissue Eng Regen Med 2016; 10: 261–270.

11. Zhang J, An Y, Gao LN, et al. The effect of aging on the pluripotential capacity and regenerative potential of human periodontal ligament stem cells. Biomaterials 2012; 33: 6974–6986.

12. Collado-González M, Pecci-Lloret MP, García-Bernal D, et al. Biological effects of silk fibroin 3D scaffolds on stem cells from human exfoliated deciduous teeth (SHEDs). Odontology 2018; 106: 125–134.

13. Rezai RM, Liu D, He H, et al. The role of dentin matrix protein 1 (DMP1) in regulation of osteogenic differentiation of rat dental follicle stem cells (DFSCs). Arch Oral Biol 2015; 60: 546–556.

14. Sonoyama W, Liu Y, Fang D, et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 2006; 1: e79.

15. Sonoyama W, Liu Y, Yamaza T, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 2008; 34: 166–171.

16. Huang GT, Gronthos S and Shi S. Mesenchymal stem cells derived from dental tissues vs those from other sources: their biology and role in regenerative medicine. J Dent Res 2009; 88: 792–806.

17. Ding G, Liu Y, An Y, et al. Suppression of T cell proliferation by root apical papilla stem cells in vitro. Cells Tissues Organs 2010; 191: 357–364.

18. Abe S, Yamaguchi S, Watanabe A, et al. Hard tissue regeneration capacity of apical pulp derived cells (APDCs) from human tooth with immature apex. Biochem Biophys Res Commun 2008; 371: 90–93.

19. Yam GH, Peh GS, Singhal S, et al. Dental stem cells: a future asset of ocular cell therapy. Expert Rev Mol Med 2015; 17: e20.

20. De Becker A, Van Hummelen P, Bakkus M, et al. Migration of culture-expanded human mesenchymal stem cells through bone marrow endothelium is regulated by matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-3. Haematologica 2007; 92: 440–449.

21. Rombouts WJ and Ploemacher RE. Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia 2003; 17: 160–170.

22. Hung SC, Pochampally RR, Hsu SC, et al. Short-term exposure of multipotent stromal cells to low oxygen increases their expression of CX3CR1 and CXCR4 and their engraftment in vivo. PLoS One 2007; 2: e416.

23. Lee SH, Lee YJ, Song CH, et al. Role of FAK phosphorylation in hypoxia-induced hMSCS migration: involvement of VEGF as well as MAPKS and eNOS pathways. Am J Physiol Cell Physiol 2010; 298: C847–C856.

24. Kallmeyer K and Michael SP. Homing properties of mesenchymal stromal cells. Expert Opin Biol Ther 2015; 15: 477–479.

25. Togel F, Weiss K, Yang Y, et al. Vasculotropic, paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. Am
26. Maleszewksa M, Vanchin B and Harmsen MC. The decrease in histone methyltransferase EZH2 in response to fluid shear stress alters endothelial gene expression and promotes quiescence. *Angiogenesis* 2016; 19: 9–24.

27. Tan JZ, Yan Y, Wang XX, et al. EZH2: biology, disease, and structure-based drug discovery. *Acta Pharmacologica Sinica* 2014; 35: 161–174.

28. Lv YF, Yan GN, Meng G, et al. Enhancer of zeste homolog 2 silencing inhibits tumor growth and lung metastasis in osteosarcoma. *Sci Rep* 2015; 5: 12999.

29. Liu L, Xu Z, Zhong L, et al. Enhancer of zeste homolog 2 (EZH2) promotes tumour cell migration and invasion via epigenetic repression of E-cadherin in renal cell carcinoma. *BJU Int* 2016; 117: 351–362.

30. Xiao Y. Enhancer of zeste homolog 2: a potential target for tumor therapy. *Int J Biochem Cell Biol* 2011; 43: 474–477.

31. Sparmann A. Polycomb silencers control cell fate, development and cancer. *Nat Rev Cancer* 2006; 6: 846–856.

32. LaFave LM, Bequelin W, Koche R, et al. Loss of BAP1 function leads to EZH2-dependent transformation. *Nat Med* 2015; 21: 1344–1349.

33. Bitler BG, Aird KM, Garipov A, et al. Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. *Nat Med* 2015; 21: 231–238.

34. Min J, Zaslavsky A, Fedele G, et al. An oncogene-tumor suppressor cascade drives metastatic prostate cancer by coordinately activating Ras and nuclear factor-kappaB. *Nat Med* 2010; 16: 286–294.

35. Chou RH, Yu YL and Hung MC. The roles of EZH2 in cell lineage commitment. *Am J Transl Res* 2011; 3: 243–250.

36. Hui T, A P, Zhao Y, et al. EZH2, a potential regulator of dental pulp inflammation and regeneration. *J Endod* 2014; 40: 1132–1138.

37. Hui T, A P, Zhao Y, et al. EZH2 regulates dental pulp inflammation by direct effect on inflammatory factors. *Arch Oral Biol* 2018; 85: 16–22.

38. Strojny C, Boyle M, Bartholomew A, et al. Interferon gamma-treated dental pulp stem cells promote human mesenchymal stem cell migration in vitro. *J Endod* 2015; 41: 1259–1264.

39. Liu L, Luo Q, Sun J, et al. Chromatin organization regulated by EZH2-mediated H3K27me3 is required for OPN-induced migration of bone marrow-derived mesenchymal stem cells. *Int J Biochem Cell Biol* 2018; 96: 29–39.

40. Ding G, Liu Y, Wang W, et al. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. *Stem Cells* 2010; 28: 1829–1838.

41. Yang H, Fan J, Cao Y, et al. Distal-less homeobox 5 promotes the osteo-/dentinogenic differentiation potential of stem cells from apical papilla by activating histone demethylase KDM4B through a positive feedback mechanism. *Exp Cell Res* 2019; 374: 221–230.

42. Wang L, Yang H, Lin X, et al. KDM1A regulated the osteo/dentinogenic differentiation process of the stem cells of the apical papilla via binding with PLOD2. *Cell Prolif* 2018; 51: e12459.

43. Al-Maghrebi M, Kehinde EO, Al-Mulla F, et al. The effect of prostate tissue inflammation on the expression of enhancer of zeste homolog 2 ribonucleic acid. *Ann Saudi Med* 2012; 32: 262–268.

44. Chou RH, Chiu L, Yu YL, et al. The potential roles of EZH2 in regenerative medicine. *Cell Transplant* 2015; 24: 313–317.

45. Palacios D, Mozzetta C, Consalvi S, et al. TNF/p38a/polycomb signaling to Pax7 locus in satellite cells links inflammation to the epigenetic control of muscle regeneration. *Cell Stem Cell* 2010; 7: 455–469.

46. Lu C, Han HD, Mangala LS, et al. Regulation of tumor angiogenesis by EZH2. *Cancer Cell* 2010; 18: 185–197.

47. Xiao X, Senavirathna LK, Gou X, et al. EZH2 enhances the differentiation of fibroblasts into myofibroblasts in idiopathic pulmonary fibrosis. *Physiol Rep* 2016; 4: pii: e12915.

48. Zhang Q, Dong P, Liu X, et al. Enhancer of zeste homolog 2 (EZH2) induces epithelial-mesenchymal transition in endometriosis. *Sci Rep* 2017; 7: 6804.
49. Naderi-Meshkin H, Bahrami AR, Bidkhori HR, et al. Strategies to improve homing of mesenchymal stem cells for greater efficacy in stem cell therapy. *Cell Biol Int* 2015; 39: 23–34.

50. De Becker A and Riet IV. Homing and migration of mesenchymal stromal cells: how to improve the efficacy of cell therapy? *World J Stem Cells* 2016; 8: 73–87.

51. Sohni A and Verfaillie CM. Mesenchymal stem cells migration homing and tracking. *Stem Cells Int* 2013; 2013: 130763.

52. Pease JE and Williams TJ. The attraction of chemokines as a target for specific anti-inflammatory therapy. *Br J Pharmacol* 2006; 147: S212–S221.

53. Bobis-Wozowicz S, Miekus K, Wybieralska E, et al. Genetically modified adipose tissue-derived mesenchymal stem cells over-expressing CXCR4 display increased motility, invasiveness, and homing to bone marrow of NOD/SCID mice. *Exp Hematol* 2011; 39: 686–696.e684.

54. Huang J, Zhang Z, Guo J, et al. Genetic modification of mesenchymal stem cells overexpressing CCR1 increases cell viability, migration, engraftment, and capillary density in the injured myocardium. *Circ Res* 2010; 106: 1753–1762.

55. Zhu J, Zhou Z, Liu Y, et al. Fractalkine and CX3CR1 are involved in the migration of intravenously grafted human bone marrow stromal cells toward ischemic brain lesion in rats. *Brain Res* 2009; 1287: 173–183.

56. Zhang Y, Zheng J, Zhou Z, et al. Fractalkine promotes chemotaxis of bone marrow-derived mesenchymal stem cells towards ischemic brain lesions through Jak2 signaling and cytoskeletal reorganization. *FEBS J* 2015; 282: 891–903.

57. Kroez KL, Jurgens WJ, Doulabi BZ, et al. Chemokine-mediated migration of skin-derived stem cells: predominant role for CCL5/RANTES. *J Invest Dermatol* 2009; 129: 1569–1581.

58. Liu N, Tian J, Cheng J, et al. Migration of CXCR4 gene modified bone marrow derived mesenchymal stem cells to the acute injured kidney. *J Cell Biochem* 2013; 114: 2677–2689.

59. Kumar S and Ponnazhagan S. Bone homing of mesenchymal stem cells by ectopic alpha 4 integrin expression. *FASEB J* 2007; 21: 3917–3927.

60. Alsayed Y, Ngo H, Runnels J, et al. Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)-dependent migration and homing in multiple myeloma. *Blood* 2007; 109: 2708–2717.

61. Lin MN, Shang DS, Sun W, et al. Involvement of PI3K and ROCK signaling pathways in migration of bone marrow-derived mesenchymal stem cells through human bone marrow microvascular endothelial cell monolayers. *Brain Res* 2013; 1513: 1–8.

62. Xie J, Wang W, Si JW, et al. Notch signaling regulates CXCR4 expression and the migration of mesenchymal stem cells. *Cell Immunol* 2013; 281: 68–75.

63. Farges JC, Keller JF, Carrouel F, et al. Odontoblasts in the dental pulp immune response. *J Exp Zool B Mol Dev Evol* 2009; 312B: 425–436.

64. Wang P, Yang X, Xu W, et al. Integrating individual functional moieties of CXCL10 and CXCL11 into a novel chimeric chemokine leads to synergistic antitumor effects: a strategy for chemokine-based multi-target-directed cancer therapy. *Cancer Immunol Immunother* 2010; 59: 1715–1726.

65. Wang Y, Xu H, Si L, et al. MiR-206 inhibits proliferation and migration of prostate cancer cells by targeting CXCL11. *Prostate* 2018; 78: 479–490.

66. Kalwitz G, Andreas K, Endres M, et al. Chemokine profile of human serum from whole blood: migratory effects of CXCL10 and CXCL11 on human mesenchymal stem cells. *Connect Tissue Res* 2010; 51: 113–122.

67. Liang K, Liu Y, Eer D, et al. High CXC chemokine ligand 16 (CXCL16) expression promotes proliferation and metastasis of lung cancer via regulating the NF-kB pathway. *Med Sci Monit* 2018; 24: 405–411.

68. Smith H, Whittall C and Weksler B. Chemokines stimulate bidirectional migration of human mesenchymal stem cells across bone marrow endothelial cells. *Stem Cells Dev* 2012; 21: 476–486.
69. Liu Q, Li A, Tian Y, et al. The CXCL8-CXCR1/2 pathways in cancer. *Cytokine Growth Factor Rev* 2016; 31: 61–71.

70. Kim SM, Kim DS, Jeong CH, et al. CXC chemokine receptor 1 enhances the ability of human umbilical cord blood-derived mesenchymal stem cells to migrate toward gliomas. *Biochem Biophys Res Commun* 2011; 407: 741–746.

71. Ratajczak MZ, Kucia M, Jadczyk T, et al. Pivotal role of paracrine effects in stem cell therapies in regenerative medicine: can we translate stem cell-secreted paracrine factors and microvesicles into better therapeutic strategies? *Leukemia* 2012; 26: 1166–1173.

72. Qazi TH, Mooney DJ and Duda GN. Biomaterials that promote cell-cell interactions enhance the paracrine function of MSCs. *Biomaterials* 2017; 140: 103–114.

73. Zhong W, Tong Y, Li Y, et al. Mesenchymal stem cells in inflammatory microenvironment potently promote metastatic growth of cholangiocarcinoma activating Akt/NF-κB signaling by paracrine CCL5. *Oncotarget* 2017; 8: 73693–73704.

74. Hiwatashi N, Bing R and Kraja I. Stem cell-mediated paracrine signaling alters fibroplasia in human vocal fold fibroblasts in vitro. *Ann Otol Rhinol Laryngol* 2017; 126: 581–588.