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Modulation of Osteogenic Differentiation in Human Dental Pulp Stem Cells by EphrinB2 Signaling

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Abstract: *Current treatments for dental pulp diseases, such as root canal therapy, fail to regenerate functional pulp tissue, highlighting the need for innovative regenerative strategies. Human dental pulp stem cells (hDPSCs) hold significant potential for pulp regeneration due to their multipotency, but the molecular pathways governing their differentiation remain poorly understood. This study investigates the role of EphrinB2 signaling in regulating osteogenic differentiation of hDPSCs and identifies EphB4 as its key mediator. Using lentiviral overexpression of EphrinB2 combined with a periodontal ligament stem cell-derived decellularized extracellular matrix (PDLSC-DECM) scaffold, we demonstrated that EphrinB2 significantly enhances early osteogenic differentiation, as evidenced by upregulated expression of RUNX2, BMP2, and IGF, alongside increased alkaline phosphatase (ALP) activity. Strikingly, inhibition of EphB4 (via TNYL-RAW peptide) suppressed osteogenesis, whereas EphB2 inhibition had no effect, indicating EphB4-specific regulation. Notably, late-stage mineralization markers (OCN, DSPP, DMP1) remained unaffected, suggesting EphrinB2-EphB4 signaling primarily drives early osteogenic commitment. These findings contrast with reports in other cell types where EphB2 dominates, underscoring cell type-specific receptor usage. Our work establishes EphrinB2-EphB4 as a critical axis for hDPSC osteogenesis and provides a scaffold-integrated strategy to advance dental pulp regeneration. This study lays the foundation for EphrinB2-targeted therapies to overcome the limitations of conventional dental treatments and promote functional tissue repair.*

Keywords: *Osteogenic Differentiation, EphrinB2 Signaling, Human Dental Pulp Stem Cells (hDPSCs), Periodontal Ligament Stem Cells (PDLSC), Extracellular Matrix (DECM)*

1. Introduction

Dental pulp diseases, such as irreversible pulpitis and pulpal necrosis, are common conditions that often result from bacterial infections, trauma, or untreated caries [1]. These conditions lead to inflammation, degeneration, and, in severe cases, complete necrosis of the dental pulp [2]. If left untreated, they can cause persistent pain, tooth loss, and even the spread of infection to surrounding tissues [3]. Current treatments like root canal therapy (RCT) and tooth extraction aim to relieve symptoms and preserve tooth structure, but they fall short in addressing pulp regeneration [4]. While RCT can eliminate infected tissue, it does not promote healing or restore pulp functionality. In more advanced cases, tooth extraction is required, leading to permanent tooth loss and the need for prosthetic solutions. These limitations underscore the need for innovative approaches that can regenerate damaged pulp tissue and offer more durable, restorative solutions [5].

Human dental pulp stem cells (hDPSCs) have gained considerable attention in regenerative medicine due to their exceptional ability to differentiate into various cell types, including odontoblasts, osteoblasts, and chondrocytes [6]. These stem cells are found within the dental pulp, a tissue located in the center of the tooth, and exhibit self-renewal and multipotency—characteristics that make them ideal candidates for tissue repair and regeneration [7]. In particular, DPSCs are of great interest for dental pulp regeneration, as they can differentiate into odontoblast-like cells to generate dentin and repair pulp tissue [1]. Additionally, DPSCs can be easily isolated from extracted teeth, offering a less invasive and readily available source of stem cells for clinical applications [8]. Despite their promising regenerative potential, the clinical use of DPSCs is hindered by a limited understanding of the complex molecular signaling pathways that regulate their differentiation, particularly into functional cell types that can effectively repair damaged pulp tissue [9].

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EphrinB2 is a member of the Eph receptor tyrosine kinase family and plays a crucial role in various cellular processes, including cell adhesion, migration, and differentiation [10]. It functions through binding to Eph receptors on adjacent cells, initiating bidirectional signaling that regulates cellular responses in development, tissue homeostasis, and regeneration [11]. Recent studies have highlighted the involvement of EphrinB2 in the differentiation of mesenchymal stem cells, particularly in the context of osteogenesis and angiogenesis [12]. In bone tissue, EphrinB2 signaling has been shown to influence osteoblast differentiation and bone remodeling, suggesting its potential as a regulator of osteogenic processes [13]. However, the specific role of EphrinB2 signaling in regulating osteogenic differentiation in hDPSCs remains poorly understood. Despite some evidence suggesting that EphrinB2 may modulate differentiation in various stem cell populations, its function in dental pulp regeneration and osteogenic differentiation of DPSCs has yet to be fully explored. This knowledge gap limits our ability to harness EphrinB2-based therapies for pulp regeneration and bone tissue engineering.

This study aims to investigate the role of EphrinB2 signaling in promoting the osteogenic differentiation of hDPSCs through the overexpression of EphrinB2. To facilitate osteogenesis, periodontal ligament stem cell (PDLSC)-derived extracellular matrix (DECM) was employed as a scaffold. The upregulation of osteogenic markers, along with enhanced alkaline phosphatase (ALP) activity, demonstrated that EphrinB2 positively regulates osteogenic differentiation of hDPSCs in the presence of PDLSC-DECM. The primary goal of this study is to elucidate the molecular mechanisms by which EphrinB2 modulates osteogenesis, thereby advancing regenerative strategies for dental and skeletal tissue repair. The findings may lay the groundwork for EphrinB2-based therapeutic approaches in dental pulp regeneration, opening new avenues for treating pulp diseases and promoting bone tissue engineering.

2. Methods

2.1 Cell Culture

hDPSCs were isolated from the extracted teeth of healthy adult patients following protocols approved by the ethics committee of Shenzhen Longgang ENT Hospital. Briefly, the dental pulp was harvested from freshly extracted third molars, and the tissue was enzymatically digested using a mixture of collagenase type I and dispase (Sigma-Aldrich). The resulting single-cell suspension was plated in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin-streptomycin (Gibco). Cells were cultured at 37°C in a humidified atmosphere with 5% CO_2, and the medium was changed every 2–3 days. After reaching 70–80% confluence, hDPSCs were passaged using 0.25% trypsin-EDTA (Gibco) and subcultured at a 1:3 ratio. PDLSCs were also isolated from extracted healthy teeth, following similar procedures as for hDPSCs. The periodontal ligament tissue was gently separated from the root surface, and cells were harvested by enzymatic digestion using collagenase type I and hyaluronidase (Sigma-Aldrich). PDLSCs were cultured under the same conditions as hDPSCs, maintaining similar subculture and passaging protocols.

2.2 Lentivirus Transduction

To overexpress EphrinB2 in hDPSCs, lentiviral vectors carrying the EphrinB2 gene were generated and used for transduction. The Lenti-EphrinB2 and control lentiviral vectors (empty vector) were purchased from a commercial supplier (GeneChem) and packaged in HEK293T cells. Briefly, HEK293T cells were co-transfected with the lentiviral plasmid and packaging vectors (psPAX2 and pMD2.G) using lipofectamine 3000 (Invitrogen). The culture medium was replaced 6 hours post-transfection, and lentivirus-containing supernatant was collected 48 hours after transfection. The harvested viral supernatant was filtered through a 0.45 μm filter to remove cell debris and concentrated by ultracentrifugation (50,000 g for 2 hours at 4°C). For transduction, hDPSCs were plated at a density of 1×10^5 cells per well in a 6-well plate and allowed to adhere overnight. The concentrated lentivirus was added to the culture medium with polybrene (8 μg/mL, Sigma-Aldrich) to enhance viral transduction efficiency. The cells were incubated with the virus for 24 hours, after which the medium was replaced with fresh culture medium. After 48 hours, transduced hDPSCs were selected using puromycin (2 μg/mL) for 3–5 days to obtain stable EphrinB2-overexpressing cells. The efficiency of transduction was confirmed by Western blotting to detect EphrinB2 expression at the protein level.

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2.3 Derivation of Decellularized Matrices from PDLSC

DECM was derived from confluent cell sheets of PDLSC (passages 5–10) following a previously published protocol [14, 15]. Briefly, PDLSC were cultured to confluence in 6-well plates in the presence of 50 μg/mL L-ascorbic acid for at least 1 week prior to matrix derivation. Once the cells reached confluency, the cell sheets were rinsed with PBS and treated with a solution containing 20 mM NH_4OH and 0.5% Triton X-100 for 5 minutes at 37°C. The remaining DECM in the wells was then rinsed again with PBS, followed by DNase treatment (1 hour at 37°C) to remove any remaining cellular DNA. After the DNase treatment, the matrices were thoroughly rinsed with deionized water, air-dried, and stored at 4°C until use in subsequent experiments.

2.4 Osteogenic Induction

To induce osteogenic differentiation, EphrinB2-overexpressing hDPSCs and control hDPSCs (passages 10–15) were seeded at a density of 300,000 cells per well in 6-well plates containing PDLSC-DECM. The cells were cultured in a non-inducing growth medium for 24 hours to allow for adhesion and the formation of a 70–80% confluent monolayer. After 24 hours, the medium was replaced with osteogenic induction medium, consisting of α-MEM (Gibco) supplemented with 10% FBS, 10^{-8} M dexamethasone (Sigma-Aldrich), 10 mM β-glycerophosphate (Sigma-Aldrich), 50 μg/mL ascorbic acid (Sigma-Aldrich), and 1% penicillin-streptomycin (Gibco). The cells were cultured in the osteogenic induction medium for up to 21 days, with medium changes every 3–4 days.

2.5 Real-time Quantitative Polymerase Chain Reaction (qRT-PCR)

Table 1: The sequences of primers used in qRT-PCR

RUNX2	Forward	TCTTAGAACAAATTCTGCCCTTT
RUNX2	Reverse	TGCTTTGGTCTTGAAATCACA
BMP2	Forward	TTCCACCATGAAGAATCTTTGGA
BMP2	Reverse	CCTGAAGCTCTGCTGAGGTGAT
PTH	Forward	CCTCGTTGACACCTGGAAGAG
PTH	Reverse	CAGATCACGTCATCGCACAAC
OCN	Forward	CTACCTGTATCAATGGCTGGG
OCN	Reverse	GGATTGAGCTCACACACCT
DSPP	Forward	TTTGGGCAGTAGCATGGGC
DSPP	Reverse	CCATCTTGGGTATTCTCTTGCCT
DMP1	Forward	CTCCGAGTTGGACGATGAGG
DMP1	Reverse	TCATGCCTGCACTGTTCATTC
BMP7	Forward	CATGGCCCAGTGCCTA
BMP7	Reverse	TACGGAAGGCTGACT
IGF	Forward	CGAAGTGAGACTTCCA
IGF	Reverse	CTCGATTACTGCATTGG
ephrinB2	Forward	TATGCAGAACTGCGATTTCCAA
ephrinB2	Reverse	TGGGTATAGTACCAGTCCTTGTC
EphB4	Forward	CGCACCTACGAAGTGTGTGA
EphB4	Reverse	GTCCGCATCGCTCTCATAGTA
EphB2	Forward	CTTGGCATATCGTACGGT
EphB2	Reverse	TCACGGCAATCAATGCTT
β-actin	Forward	ACGTTGCTATCCAGGCTGTG
β-actin	Reverse	GGCCATCTCTTGCTCGAAGT

To assess osteogenic differentiation, qRT-PCR was performed to quantify the expression of key osteogenic markers. Total RNA was isolated using the RNeasy Plus Mini Kit (Qiagen), and the concentration and quality of RNA were evaluated using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Only RNA samples with an A260/A280 ratio > 1.8 were used for subsequent analysis. Reverse transcription was carried out using the SuperScript VILO Master Mix (Life Technologies) to generate complementary DNA (cDNA) from 1 μg of total RNA. qRT-PCR was performed using the SYBR Select Master Mix (Applied Biosystems) on a StepOne Real-Time PCR System (Applied Biosystems). The amplification reactions were prepared in a total volume of 20 μL, containing 10 μL SYBR Green Supermix, 0.5 μM forward and reverse primers (listed in Table 1), and 1 μL cDNA template.

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The amplification program consisted of initial denaturation at 95°C for 2 minutes, followed by 40 cycles of 95°C for 3 seconds, and 60°C for 30 seconds. A melting curve analysis was performed to verify the specificity of the PCR products. Relative gene expression was calculated using the ΔΔCt method and normalized against GAPDH as an internal housekeeping gene. The expression of osteogenic markers in EphrinB2-overexpressing hDPSCs was compared to that in control cells cultured on PDLSC-DECM.

2.6 Assay of ALP Activity

ALP activity was measured as an indicator of osteogenic differentiation. After osteogenic induction, the cells were washed with PBS and lysed using ALP lysis buffer (50 mM Tris-HCl, pH 9.0, 1 mM $MgCl_2$). The cell lysates were then centrifuged at 12,000 × g for 10 minutes at 4°C, and the supernatant was collected for ALP activity measurement. ALP activity was assessed using the p-nitrophenyl phosphate (pNPP) assay. The reaction mixture consisted of 100 µL of cell lysate, 100 µL of pNPP substrate (Sigma-Aldrich), and 100 µL of Alkaline Phosphatase Assay Buffer (Sigma-Aldrich). The mixture was incubated at 37°C for 30 minutes, and the reaction was terminated by adding 100 µL of 0.2 M NaOH. Absorbance was measured at 405 nm. The results were normalized to the total protein content, which was determined using the BCA Protein Assay Kit (Thermo Fisher Scientific).

2.7 Statistical Analysis

Data were expressed as the mean ± standard deviation (SD) from at least three independent biological replicates. Statistical differences between experimental groups were assessed by Student's t-test using SPSS 19.0 Statistics Software (SPSS Inc). A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Effect of EphrinB2-Fc on the osteogenic differentiation of hDPSCs

To investigate the role of EphrinB2 in the osteogenic differentiation of hDPSCs, cells were cultured on PDLSC-derived DECM in osteogenic induction medium, with or without EphrinB2-Fc stimulation. qRT-PCR analysis (Figure 1) revealed that the expression levels of RUNX2, BMP2, BMP7, PTH, and IGF were significantly upregulated in EphrinB2-Fc-treated hDPSCs compared to the control group ($p < 0.05$), whereas OCN, DSPP, and DMP1 showed no significant differences between the two groups. Cell proliferation analysis using the CCK-8 assay (Figure 2) demonstrated that the EphrinB2-Fc-treated group exhibited slower proliferation rates than the control group on days 4, 6, and 8, suggesting that EphrinB2-Fc may modulate cell growth during differentiation. In contrast, ALP activity assays (Figure 2) revealed a significant increase in alkaline phosphatase activity in EphrinB2-Fc-treated hDPSCs, indicating enhanced osteogenic differentiation. Additionally, qRT-PCR analysis (Figure 3) indicated a significant downregulation of EphB2 and EphB4 in hDPSCs exposed to EphrinB2-Fc, while the expression of endogenous EphrinB2 remained unchanged. These findings suggest that EphrinB2-Fc enhances osteogenic differentiation by upregulating key osteogenic markers and ALP activity, while also modulating cell proliferation and EphB receptor expression in hDPSCs.

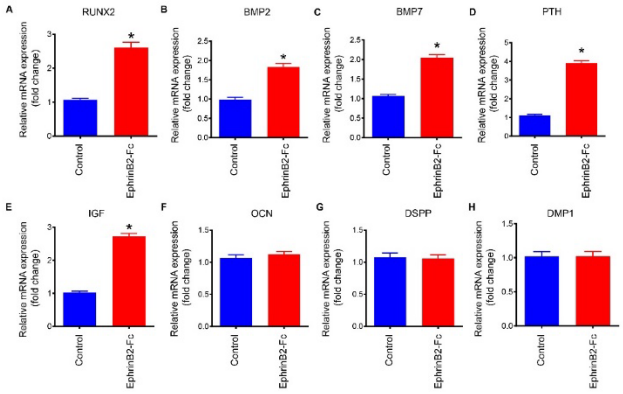

Figure 1: EphrinB2-Fc enhances osteogenic marker expression in hDPSCs.

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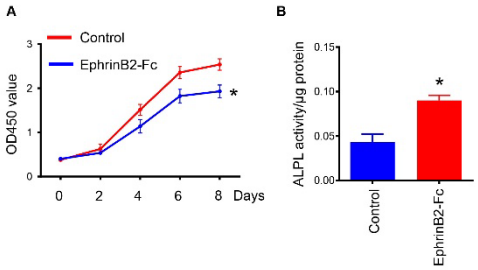

Figure 2: EphrinB2-Fc reduces hDPSC proliferation but increases ALP activity.

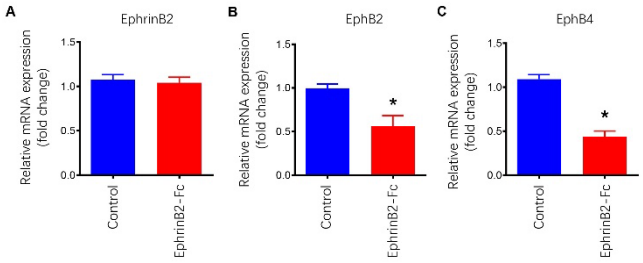

Figure 3: EphrinB2-Fc downregulates EphB2 and EphB4 in hDPSCs.

3.2 Regulation of osteogenic differentiation in hDPSCs by TNYL-RAW and SNEW peptides

To further investigate the role of EphB2 and EphB4 in EphrinB2-mediated osteogenic differentiation, hDPSCs were treated with specific inhibitory peptides targeting these receptors at a concentration of 100 μM. TNYL-RAW, which selectively binds to EphB4, was used alongside its control peptide, SCR-WTL (Fig. 4). Treatment with TNYL-RAW significantly suppressed the expression of key osteogenic markers, including RUNX2, BMP2, BMP7, PTH, and IGF, compared to the control group, indicating that EphB4 signaling contributes to osteogenic differentiation. In contrast, administration of SNEW, a peptide that specifically targets EphB2, along with its control peptide, SCR-EPQ, did not result in any significant changes in the expression of osteogenic markers (Fig. 5). These findings suggest that EphB4 plays a more prominent role than EphB2 in regulating the osteogenic differentiation of hDPSCs.

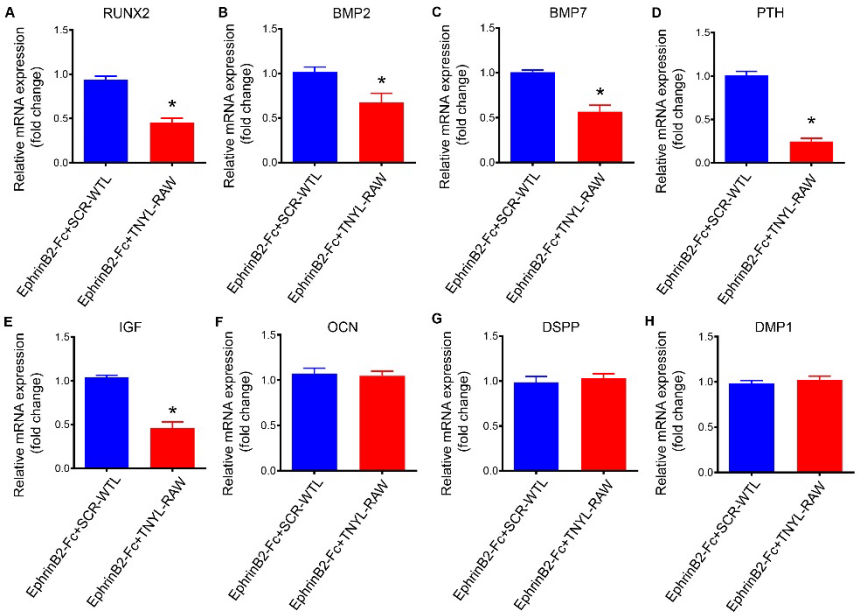

Figure 4: Inhibition of EphB4 by TNYL-RAW reduces osteogenic marker expression in hDPSCs.

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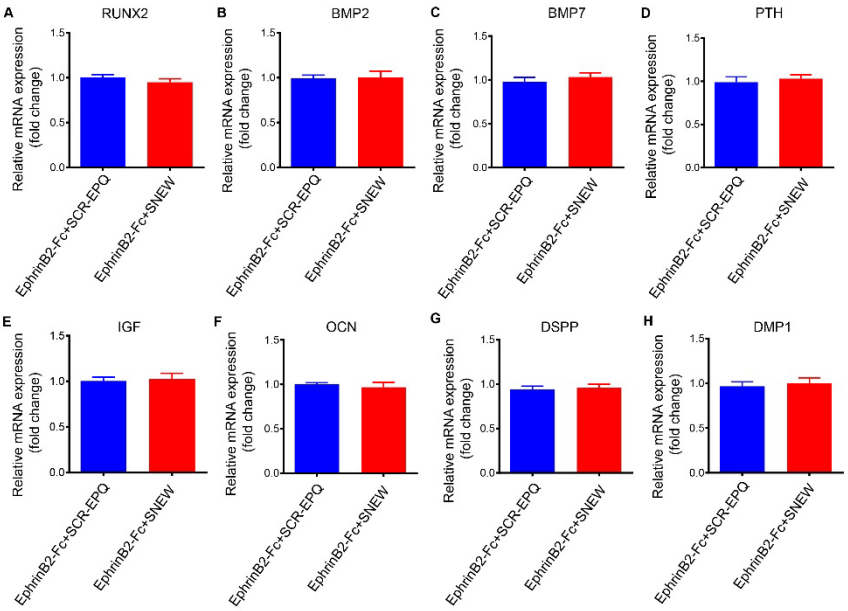

Figure 5: Inhibition of EphB2 by SNEW has no significant effect on osteogenic differentiation of hDPSCs.

3.3 EphrinB2 overexpression promoted osteogenic differentiation of hDPSCs

To further elucidate the role of EphrinB2 in osteogenic differentiation, hDPSCs were genetically modified to overexpress EphrinB2. Western blot analysis (Fig. 6A) and qRT-PCR (Fig. 6B) confirmed the successful overexpression of EphrinB2 in the transfected cells. To evaluate the impact of EphrinB2 overexpression on cell proliferation, a CCK-8 assay was performed (Fig. 6C), revealing a slightly reduced proliferation rate in the EphrinB2-overexpressing hDPSCs compared to the control group. Additionally, ALPL activity, a key marker of early osteogenic differentiation, was significantly elevated in the EphrinB2-overexpressing group (Fig. 6D), suggesting enhanced osteogenic potential. Further validation of osteogenic differentiation was conducted through qRT-PCR analysis of specific osteogenic markers (Fig. 7). The results demonstrated a significant upregulation of RUNX2, BMP2, BMP7, PTH, and IGF in the EphrinB2-overexpressing hDPSCs compared to the control group, while no significant differences were observed in the expression of OCN, DSPP, and DMP1. These findings indicate that EphrinB2 positively regulates the osteogenic differentiation of hDPSCs, further supporting its potential role in regenerative applications.

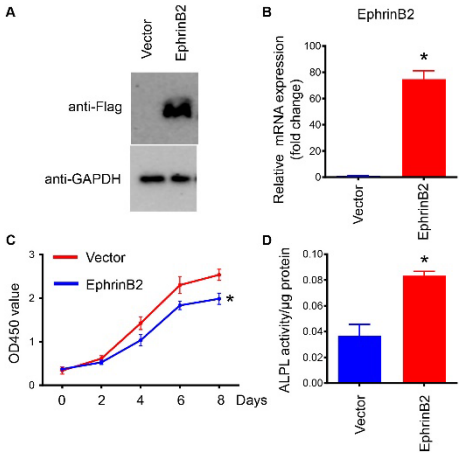

Figure 6: EphrinB2 overexpression in hDPSCs enhances osteogenic differentiation and reduces proliferation.

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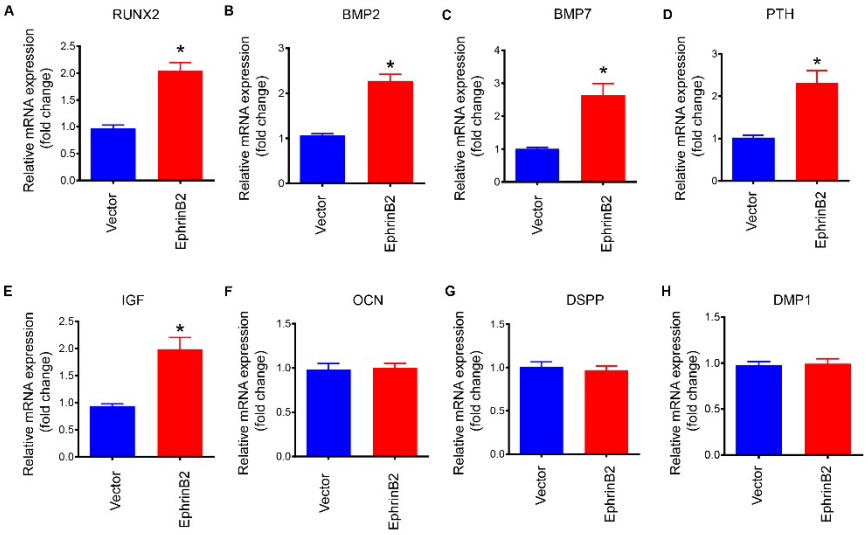

Figure 7: Upregulation of osteogenic markers in EphrinB2-overexpressing hDPSCs.

3.4 EphB4-Fc influence on hDPSC osteogenesis

To investigate the effect of EphB4-Fc on the osteogenic differentiation of hDPSCs, qRT-PCR was performed to assess the expression of osteogenic markers (Fig. 8). The results demonstrated a significant upregulation of RUNX2, BMP2, BMP7, PTH, and IGF in the EphB4-Fc-treated group compared to the control, whereas no significant differences were observed in the expression levels of OCN, DSPP, and DMP1. These findings suggest that EphB4-Fc enhances the osteogenic differentiation of hDPSCs by promoting the expression of key osteogenic regulators. To further examine the molecular response of hDPSCs to EphB4-Fc treatment, qRT-PCR analysis was performed to assess the expression levels of EphrinB2, EphB2, and EphB4 (Fig. 9). The results showed no significant differences in the expression of any of these genes between the EphB4-Fc-treated and control groups. These findings indicate that the pro-osteogenic effects of EphB4-Fc are likely mediated through downstream signaling mechanisms rather than direct modulation of EphrinB2, EphB2, or EphB4 gene expression.

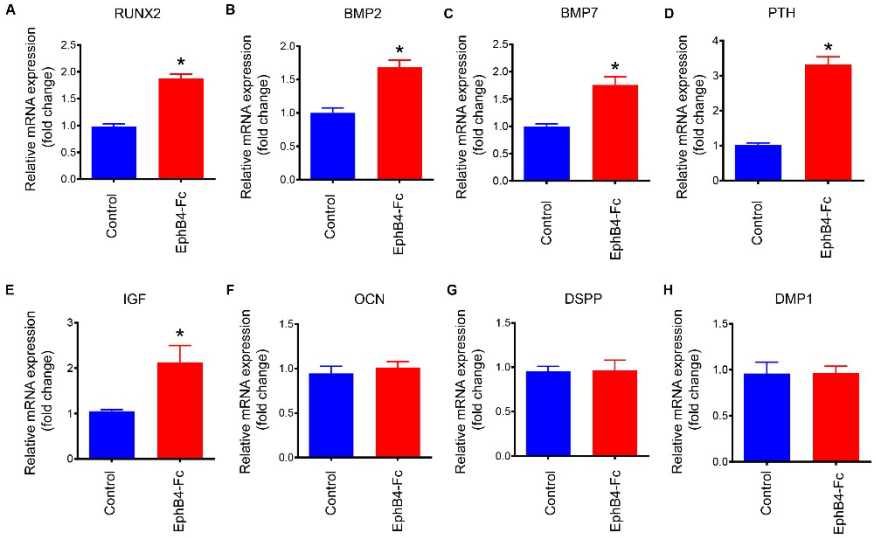

Figure 8: EphB4-Fc promotes osteogenic differentiation of hDPSCs by upregulating key osteogenic markers.

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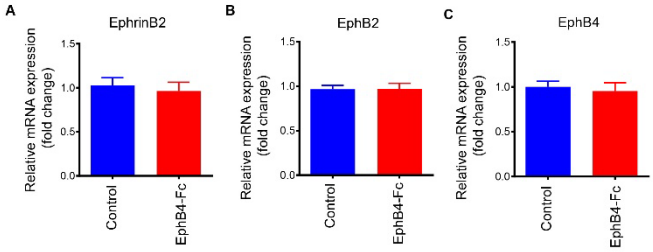

Figure 9: EphB4-Fc treatment does not alter the expression of EphrinB2, EphB2, or EphB4 in hDPSCs.

4. Discussions

In this study, we demonstrated that EphrinB2 signaling plays a pivotal role in promoting the osteogenic differentiation of hDPSCs. The results demonstrated that EphrinB2-Fc significantly upregulated key osteogenic markers, including RUNX2, BMP2, BMP7, PTH, and IGF, while also enhancing ALP activity and reducing cell proliferation. Notably, inhibition of EphB4 with TNYL-RAW led to a marked suppression of osteogenic marker expression, whereas inhibition of EphB2 with SNEW had no significant effect, indicating that EphB4 plays a more prominent role than EphB2 in mediating osteogenic differentiation. Overexpression of EphrinB2 further enhanced osteogenic differentiation, reinforcing its regulatory role. Furthermore, EphB4-Fc stimulation promoted osteogenic marker expression without altering the expression levels of EphrinB2, EphB2, or EphB4, suggesting that its effects are mediated through downstream signaling pathways rather than direct receptor modulation. These findings provide novel insights into the molecular mechanisms underlying EphrinB2-mediated osteogenesis and highlight the potential of targeting this pathway for dental pulp regeneration and bone tissue engineering.

Previous studies have identified EphrinB2-EphB4 signaling as a key regulator of bone remodeling and osteogenesis [16]. Our findings align closely with the study by Wang et al. [17], which reported that EphrinB2 overexpression promotes osteogenic differentiation in DPSCs through EphrinB2-mediated reverse signaling. Our observation that EphrinB2 overexpression enhances osteogenic differentiation in hDPSCs directly supports the conclusions of Wang et al., who demonstrated that EphrinB2 overexpression in DPSCs activates reverse signaling pathways, leading to increased osteogenic potential and improved bone repair in a canine alveolar defect model. Both studies highlight the pivotal role of EphrinB2 in regulating osteogenesis, particularly through mechanisms involving bidirectional signaling. For example, Wang et al. observed that phosphorylated EphrinB2 (p-EphrinB2) was upregulated during osteogenic induction, a finding mirrored in our study, where EphrinB2 overexpression correlated with elevated ALP activity and osteogenic gene expression.

The findings of this study demonstrate that EphrinB2-EphB4 signaling promotes osteogenic differentiation in hDPSCs by enhancing the expression of critical transcription factors and growth factors, notably RUNX2, BMP2, and IGF. The significant increase in ALP activity further underscores the role of this signaling axis in early-stage osteogenesis [18]. Interestingly, the lack of significant changes in OCN, DSPP, and DMP1 expression suggests that EphrinB2/EphB4 signaling primarily regulates early differentiation events rather than late-stage mineralization, aligning with observations in other stem cell systems where early markers are selectively modulated during lineage commitment [19]. A key distinction of our work is the identification of EphB4, rather than EphB2, as the dominant receptor mediating EphrinB2's effects in hDPSCs. While EphB2 has been reported to play a prominent role in osteogenesis in other cell types—such as bone marrow-derived mesenchymal stem cells (BMSCs) or neural stem cells (NSCs)—our results highlight cell type-specific receptor usage [20]. For instance, studies in neural stem cells demonstrated that EphB2 activation enhances neurogenesis and cognitive function, but this receptor's role in osteogenesis remains context-dependent [20]. Similarly, in prostate cancer, EphB2 regulates lipid metabolism rather than osteogenic pathways, further emphasizing the functional diversity of Eph receptors across tissues [21]. These discrepancies may arise from differences in downstream signaling crosstalk (e.g., MAPK/ERK vs. PI3K/AKT) or interactions with lineage-specific factors such as BMP or Wnt proteins [22].

These findings highlight the potential of EphrinB2 and EphB4 signaling as therapeutic targets for bone tissue engineering and regenerative dentistry. The ability of EphrinB2 and EphB4-Fc to enhance osteogenic differentiation of hDPSCs suggests their potential application in dental pulp regeneration,

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periodontal repair, and bone defect healing. Moreover, the use of PDLSC-DECM as a scaffold provided a biomimetic microenvironment that supported osteogenic differentiation. This approach builds on previous work demonstrating the utility of decellularized matrices in tissue engineering [14, 15]. Future research should explore the feasibility of incorporating EphrinB2/EphB4-modulating biomaterials or gene-editing approaches into tissue-engineered constructs for bone regeneration.

While this study provides valuable insights, several limitations should be acknowledged. First, the study was conducted in vitro, and additional in vivo experiments are necessary to validate the osteogenic potential of EphrinB2/EphB4 signaling in a physiological environment. Second, the specific downstream signaling pathways activated by EphrinB2/EphB4 in hDPSCs remain unclear, and further mechanistic studies are needed to delineate the precise molecular interactions involved. Additionally, long-term differentiation studies should be performed to assess whether EphrinB2-EphB4 signaling contributes to late-stage mineralization. Future research should also explore combinatorial strategies involving biomaterials, gene therapy, or small-molecule modulators to enhance the regenerative potential of hDPSCs.

5. Conclusion

In conclusion, our findings demonstrate that EphrinB2 signaling, mediated primarily through EphB4, enhances the osteogenic differentiation of hDPSCs. These results provide a foundation for developing EphrinB2-based therapeutic strategies for dental and skeletal tissue regeneration. Further research is needed to translate these findings into clinical applications and to explore the broader implications of EphrinB2 signaling in regenerative medicine.

Conflict of Interest

The authors declare no relevant conflicts of interests in the publication of this article.

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References

[1] Arifin, F.A., et al., Comparison of oral health-related quality of life among endodontic patients with irreversible pulpitis and pulp necrosis using the oral health-related endodontic patient's quality of life scale. Odontology, 2024.
[2] Dahake, P.T. and S. Kothari, Microbiological Profile of Primary Teeth with Irreversible Pulpitis and Pulp Necrosis with/without Abscess and their Susceptibility to Three Antibiotics as Intracanal Medication. Int J Clin Pediatr Dent, 2023. 16(2): p. 312-320.
[3] Kokkas, A.B., et al., Irreversible but not reversible pulpitis is associated with up-regulation of tumour necrosis factor-alpha gene expression in human pulp. Int Endod J, 2007. 40(3): p. 198-203.
[4] Weitz, D., et al., Preoperative Factors Associated with Anesthesia Failure for Patients Undergoing Nonsurgical Root Canal Therapy: A National Dental Practice-Based Research Network Study. J Endod, 2021. 47(12): p. 1875-1882.
[5] Alqahtani, Q., et al., Decellularized Swine Dental Pulp Tissue for Regenerative Root Canal Therapy. J Dent Res, 2018. 97(13): p. 1460-1467.
[6] Stefanska, K., et al., Dental pulp stem cells - A basic research and future application in regenerative medicine. Biomed Pharmacother, 2024. 178: p. 116990.
[7] Nakashima, M. and H. Tanaka, Pulp Regenerative Therapy Using Autologous Dental Pulp Stem Cells in a Mature Tooth with Apical Periodontitis: A Case Report. J Endod, 2024. 50(2): p. 189-195.
[8] Alshawkani, H.A., et al., Regenerative Potential of Dental Pulp Stem Cells in Response to a Bioceramic Dental Sealer and Photobiomodulation: An In Vitro Study. J Contemp Dent Pract, 2024. 25(4): p. 313-319.
[9] Hardin, L.T., et al., Cigarette smoking exposure disrupts the regenerative potential of dental pulp stem cells. Tob Induc Dis, 2023. 21: p. 101.

ISSN 2618-1584 Vol. 7, Issue 2: 73-82, DOI: 10.25236/FMSR.2025.070211

[10] Yu, H., et al., Tensile force promotes osteogenic differentiation via ephrinB2-EphB4 signaling pathway in orthodontic tooth movement. BMC Oral Health, 2025. 25(1): p. 118.
[11] Fu, Y., et al., Imbalanced EphB4/EphrinB2 Signaling Modulates Bone Resorption in Periodontitis Induced by Porphyromonas gingivalis. ACS Infect Dis, 2024. 10(4): p. 1152-1161.
[12] Zhou, J., et al., Low-intensity pulsed ultrasound regulates osteoblast-osteoclast crosstalk via EphrinB2/EphB4 signaling for orthodontic alveolar bone remodeling. Front Bioeng Biotechnol, 2023. 11: p. 1192720.
[13] Zhu, S., et al., Bidirectional ephrinB2-EphB4 signaling regulates the osteogenic differentiation of canine periodontal ligament stem cells. Int J Mol Med, 2020. 45(3): p. 897-909.
[14] Decaris, M.L., et al., Transferable cell-secreted extracellular matrices enhance osteogenic differentiation. Acta Biomater, 2012. 8(2): p. 744-52.
[15] Decaris, M.L., et al., Cell-derived matrix coatings for polymeric scaffolds. Tissue Eng Part A, 2012. 18(19-20): p. 2148-57.
[16] Fan, W.B., J.N. Zhao, and N.R. Bao, [Effects of bidirectional EphB4-EphrinB2 signaling on bone remodeling]. Zhongguo Gu Shang, 2013. 26(8): p. 705-8.
[17] Wang, W., et al., EphrinB2 overexpression enhances osteogenic differentiation of dental pulp stem cells partially through ephrinB2-mediated reverse signaling. Stem Cell Res Ther, 2020. 11(1): p. 40.
[18] Agarwal, K. and R. Lamprecht, EphB2 activation in neural stem cells in the basolateral amygdala facilitates neurogenesis and enhances long-term memory. Cell Mol Life Sci, 2024. 81(1): p. 277.
[19] Heng, B.C., et al., EphrinB2 signaling enhances osteogenic/odontogenic differentiation of human dental pulp stem cells. Arch Oral Biol, 2018. 87: p. 62-71.
[20] Morales, A., et al., Loss of ephrin B2 receptor (EPHB2) sets lipid rheostat by regulating proteins DGAT1 and ATGL inducing lipid droplet storage in prostate cancer cells. Lab Invest, 2021. 101(7): p. 921-934.
[21] Liu, Y.L., et al., Spatial EGFR Dynamics and Metastatic Phenotypes Modulated by Upregulated EphB2 and Src Pathways in Advanced Prostate Cancer. Cancers (Basel), 2019. 11(12).
[22] Zhao, C., et al., Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. Cell Metab, 2006. 4(2): p. 111-21.