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Original Article

Hypoxic stem cells from apical papilla-derived exosomes enhance dental pulp stem cells migration and differentiation via mitochondrial activation

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KEYWORDS
Dental pulp stem cells (DPSCs);
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Abstract *Background/purpose:* Stem cells from apical papilla (SCAPs) and dental pulp stem cells (DPSCs) play critical roles in dental tissue regeneration. Although intercellular communication through exosomes is known to influence stem cell regulation, the effects of exosomes derived from hypoxic SCAPs on the migration and odontogenic differentiation of DPSCs have not been elucidated. This study aimed to investigate SCAPs derived exosomes mediated modulation of DPSCs biological functions under hypoxic conditions.
Materials and methods: Exosomes were obtained from SCAPs cultured under normoxic or hypoxic conditions. DPSCs were treated with these exosomes, and their migration capacity was evaluated using scratch and transwell assays. Odontogenic differentiation was assessed via gene/protein expression analysis and alizarin red / alkaline phosphatase (ALP) staining. Mitochondrial dynamics and energy metabolism were analyzed using transmission electron microscopy, seahorse assays, and Mitotracker red staining.
Results: Hypoxic SCAPs-derived exosomes exhibited comparable morphology and size to normoxic exosomes but enhanced DPSCs migration and odontogenic differentiation. Hypo-Exo promoted mitochondrial fusion in DPSCs, evidenced by increased mitofusin 2 (MFN2) / translocator of the outer mitochondrial membrane 20 (TOM20) expression and augmented mitochondrial oxygen consumption rates. Rotenone inhibition of mitochondrial metabolism abrogated Hypo-Exo-induced migration and differentiation, confirming the critical role of mitochondrial fusionmediated energy metabolism in this process.
Conclusion: Hypoxic SCAPs-derived exosomes enhance DPSCs migration and odontogenic differentiation through mitochondrial fusion. These findings reveal a novel "exosome-mitochondria" regulatory axis, providing a mechanistic basis for developing hypoxia-engineered exosome therapies in dental regenerative medicine.

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Introduction

Stem cells from apical papilla (SCAPs) and dental pulp stem cells (DPSCs), as integral components of the odontogenic mesenchymal stem cell family, exhibit considerable potential for application in dental tissue regeneration.[1,2] SCAPs are predominantly sourced from the developing apical papilla tissue of immature permanent teeth, whereas DPSCs are isolated from dental pulp tissue. Both types of stem cells exhibit multipotent differentiation potential, including osteogenic, chondrogenic, and adipogenic lineages, and possess robust proliferative capacity. Consequently, they are regarded as essential seed cells for regenerative medicine applications.[3,4] The anatomical proximity of the apical papilla and dental pulp tissues suggests potential interactive regulation between stem cells derived from these sources. Although previous research has compared the biological differences between SCAPs and DPSCs—such as SCAPs' enhanced proliferative capacity and high expression of the specific surface marker CD24, in contrast to DPSCs' superior osteogenic differentiation potential, the existence of direct intercellular communication mechanisms between these stem cells has not yet been clearly elucidated.[5,6]

In recent years, research on intercellular communication mechanisms has increasingly concentrated on nanoscale vesicles known as exosomes.[7] These membrane-bound vesicles, measuring 80—150 nm in diameter, are integral to the maintenance of tissue homeostasis and the regulation of regeneration, primarily through the delivery of bioactive components such as functional microRNAs, messenger RNAs, and signaling proteins.[8—10] one recent study has revealed that exosomes derived from SCAPs can markedly enhance the expression of angiogenesis-related genes in human umbilical vein endothelial cells (HUVECs), thereby offering a novel approach for the development of vascularized bone tissue engineering.[11] This evidence suggests that SCAPs may modulate the biological behaviors of adjacent DPSCs via an exosome-mediated paracrine mechanism.

Furthermore, in comparison to traditional stem cell transplantation therapy, exosome therapy has gained prominence as a focal point in tissue regeneration research due to its distinct advantages, such as low immunogenicity, high stability, and the ability to circumvent risks associated with cell transplantation, including tumorigenicity and immune rejection.[12—14] Importantly, the influence of microenvironmental factors, particularly hypoxia, on the secretion and function of stem cell-derived exosomes represents an expanding area of investigation with substantial implications for elucidating cellular communication and advancing therapeutic applications.[15] Hypoxia, a common characteristic of numerous pathological conditions, has been demonstrated to significantly impact the biogenesis, cargo composition, and functional roles of exosomes. For example, studies on hepatocellular carcinoma cells have revealed that hypoxic conditions induce notable changes in the expression of exosomal miRNAs, which are involved in various cancer-related pathways.[16] Similarly, alterations in exosomal miRNA content induced by hypoxia have been documented in mesenchymal stem cells (MSCs), wherein hypoxic preconditioning augments the therapeutic efficacy of MSC-derived exosomes in the context of spinal cord injury by influencing microglial polarization.[17] These investigations offer compelling evidence that hypoxia serves as a pivotal factor in exosome-mediated communication, bearing significant implications for therapeutic interventions. A more comprehensive understanding of the function of SCAPs-derived exosomes under hypoxic conditions will be essential for fully exploiting their potential in clinical applications.

Addressing the existing research gap concerning the intercellular communication between the SCAPs and DPSCs, we systematically assessed the effects of SCAP-derived exosomes on the migration and differentiation capacities of DPSCs. Moreover, this study demonstrated that SCAP-derived exosomes influenced the mitochondrial fusion process and activated the mitochondrial metabolic pathway in DPSCs under hypoxic conditions. This study not only offers a novel perspective for understanding the regulatory network of tooth-derived stem cells but also establishes an experimental foundation for the development of exosome-based dental regeneration strategies.

Materials and methods

Cell culture

Apical papilla and dental pulp tissues were obtained from healthy, undeveloped premolars extracted for orthodontic purposes in patients aged 15—18 years (n = 6) at the orthodontics department of our institution. Briefly, employing a modified tissue block enzymatic digestion protocol, tooth surfaces were rinsed thrice with PBS containing dual antibiotics.[18,19] Both tissues were carefully sectioned into fragments and subjected to enzymatic digestion using a solution comprising 3 mg/mL type I collagenase (Sigma, St. Louis, MO, USA) and 4 mg/mL trypsin (Gibco, Grand Island, NE, USA) at 37 °C with agitation for 20 min. Following digestion, cells were collected by centrifugation at 400 *g* for 5 min, resuspended in α-minimal essential medium (Gibco) supplemented with 10 % FBS, and cultured in an incubator set at 37 °C with 5 % CO_2.

Flow cytometric analysis

SCAPs and DPSCs were immunophenotyped utilizing flow cytometry in conjunction with the BD Stemflow™ hMSC

Analysis Kit (BD, Franklin Lakes, NJ, USA). The markers detected included mesenchymal stem cell-positive markers: CD73, CD90, and CD105. Additionally, hematopoietic cell-negative markers CD34 and CD45 were assessed.

Establishment of a hypoxic model

An in vitro oxygen gradient system was developed utilizing a tri-gas incubator (Thermo, Waltham, MA, USA). The normoxic group was maintained at 21 % O_2 and 5 % CO_2, while the hypoxic group was maintained at 1 % O_2 and 5 % CO_2. Upon reaching 70 % confluence, the SCAPs had their culture medium replaced with exosome-depleted fetal bovine serum (System Biosciences, San Jose, CA, USA), and the cells were subsequently treated for 48 h. Real-time monitoring of dissolved oxygen levels within the culture system was conducted using an oxygen sensor.

Exosome isolation and characterization

Upon reaching 80 % confluence, SCAPs had their medium replaced with exosome-depleted serum medium (System Biosciences). Subsequently, the cells were cultured under normoxic or hypoxic conditions for a duration of 48 h. The conditioned medium underwent sequential centrifugation at 2000*g* for 10 min and 10,000 g for 30 min at 4 °C to eliminate cellular debris. Exosomes were subsequently isolated by ultracentrifugation at 120,000 g for 2 h, resuspended in prefiltered (0.22 μm) cold PBS, and stored at −80 °C. For morphological analysis, 10 μL of the exosome suspension was applied to a copper grid, negatively stained with 2 % phosphotungstic acid, and examined using a transmission electron microscope. The particle size distribution and concentration were assessed using a nanoparticle tracking analyzer. The total protein content was quantified using the bicinchoninic acid assay, and the presence of exosomal markers Tsg101, Alix, and Calnexin was confirmed through Western blot analysis.

Exosome uptake efficiency assay

Exosomes were labeled with PKH26 fluorescent dye (Sigma) as follows: the exosome suspension was incubated with PKH26 at a final concentration of 2 μM at room temperature in the dark for 20 min. The staining process was terminated by the addition of 1 % BSA. Subsequently, free dye was eliminated through ultracentrifugation at 190,000 g for 2 h. The labeled exosomes were then washed three times with PBS and resuspended. These labeled exosomes, at a concentration of 50 μg/mL, were co-cultured with DPSCs for a duration of 12 h. Then the cells were fixed using 4 % paraformaldehyde and stained DAPI (Beyotime, Shanghai, China) for nuclear counterstaining. Internalization of the exosomes was observed using a confocal laser scanning microscope, and fluorescence intensity was quantitatively analyzed using ImageJ software.

Assessment of DPSCs odontogenic differentiation

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized using PrimeScript RT Master Mix (Takara, Kyoto, Japan). Real-time quantitative PCR (RT-qPCR) was conducted on a QuantStudio 5 system with SYBR Green PCR Master Mix (Vazyme, Nanjing, China) to measure mRNA levels of *DSPP*, *RUNX2*, and *ALP*, using *GAPDH* as the internal control. Relative expression was determined via the $2^{-\Delta\Delta Ct}$ method, with primer sequences detailed in Table 1. For protein analysis, total protein was extracted using RIPA lysis buffer (Beyotime), quantified by BCA assay, and 30 μg was resolved by SDS-PAGE. Membranes were incubated with primary antibodies against DSPP (ab272929; Abcam, Cambridge, UK), RUNX2 (ab192256; Abcam), and ALP (ab229126; Abcam), followed by ECL development and quantification with ImageJ. Osteogenic differentiation was further confirmed using Alkaline phosphatase (ALP) and alizarin red staining kits.

Mitochondrial morphology observation by transmission electron microscope

DPSCs were subjected to fixation overnight at 4 °C using a 3 % glutaraldehyde solution prepared in 0.1 M PBS. This was followed by post-fixation with 1 % osmium tetroxide for a duration of 2 h. The samples were subsequently dehydrated through a graded ethanol series and embedded in epoxy resin (Sigma). Ultra-thin sections, approximately 70 nm in thickness, were then prepared and subjected to double-staining with uranyl acetate and lead citrate. Examination was conducted using a transmission electron microscope.

Seahorse assay for energy metabolism

Cellular energy metabolism was assessed utilizing a Seahorse XFe96 analyzer (Agilent, Santa Clara, CA, USA). DPSCs were seeded at a density of 2×10^4 cells per well in Seahorse-specific plates, followed by incubation for 1 h at 37 °C. Subsequently, the cells were sequentially treated with oligomycin (1 μM), FCCP (1 μM), and rotenone/antimycin A (0.5 μM). The basal oxygen consumption rate (OCR) and maximal respiratory capacity were continuously monitored in real-time.

Table 1 The primer sequences of dentin sialophosphoprotein (*DSPP*), alkaline phosphatase (*ALP*), and runt-related transcription factor 2 (*RUNX2*) used for RT-qPCR.

Gene	Forward primers (5'-3')	Reverse primers (5'-3')
DSPP	ATATTGAGGGCTGGAATGGGGA	TTTGTGGCTCCAGCATTGTCA
ALP	GACCTCCTCGGAAGACACTC	TGAAGGGCTTCTTGTCTGTG
RUNX2	TCTTAGAACAAATTCTGCCCTTT	TGCTTTGGTCTTGAAATCACA
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

Data analysis was conducted using Wave 2.6.1 software, with results normalized to the total cellular protein content.

Mito-tracker red staining

DPSCs were incubated with 5 μM Mito-tracker red (Beyotime) for 20 min. Subsequently, the cells were washed with warm PBS to eliminate any excess dye. The stained cells were then visualized using a Nikon fluorescence microscope, with random fields captured for each experimental group.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS version 26.0. Comparisons between two groups were performed using independent samples t-tests, while comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA). A $P < 0.05$ was designated as *. Graphical representations were created using GraphPad Prism version 9.0.

Results

Isolation and identification of SCAPs and DPSCs

Primary SCAPs and DPSCs were effectively isolated from apical papilla and dental pulp tissues utilizing an enzymatic digestion technique. The primary cells demonstrated typical adherent growth properties, with cellular migration from the periphery of tissue blocks observed microscopically. Upon subculturing, the cells exhibited a uniform morphology, characterized by elongated spindle-shaped fibroblastic features (Fig. 1A). Flow cytometric analysis indicated that SCAPs and DPSCs exhibited high expression levels of mesenchymal stem cell surface markers CD73, CD90, and CD105, while hematopoietic cell markers CD34 and CD45 were not expressed (Fig. 1B).

Effect of hypoxic microenvironment on exosome secretion by SCAPs

Exosomes secreted by SCAPs under normoxic and hypoxic conditions were isolated using differential centrifugation. Transmission electron microscopy analysis demonstrated that both groups of exosomes exhibited the characteristic cup-shaped vesicular morphology (Fig. 2A). Nanoparticle tracking analysis indicated mean particle sizes of 142 nm for the normoxic exosome group (Nor-Exo) and 147 nm for the hypoxic exosome group (Hypo-Exo), with no statistically significant difference observed between the groups (Fig. 2B and C). Western blot analysis confirmed the presence of exosomal marker proteins CD9, CD63, and TSG101 in both exosome groups (Fig. 2D).[20]

Regulatory effect of hypoxic SCAPs-derived exosomes on DPSCs migration

The PKH26 fluorescent tracing demonstrated that DPSCs co-cultured with both Nor-Exo and Hypo-Exo exhibited

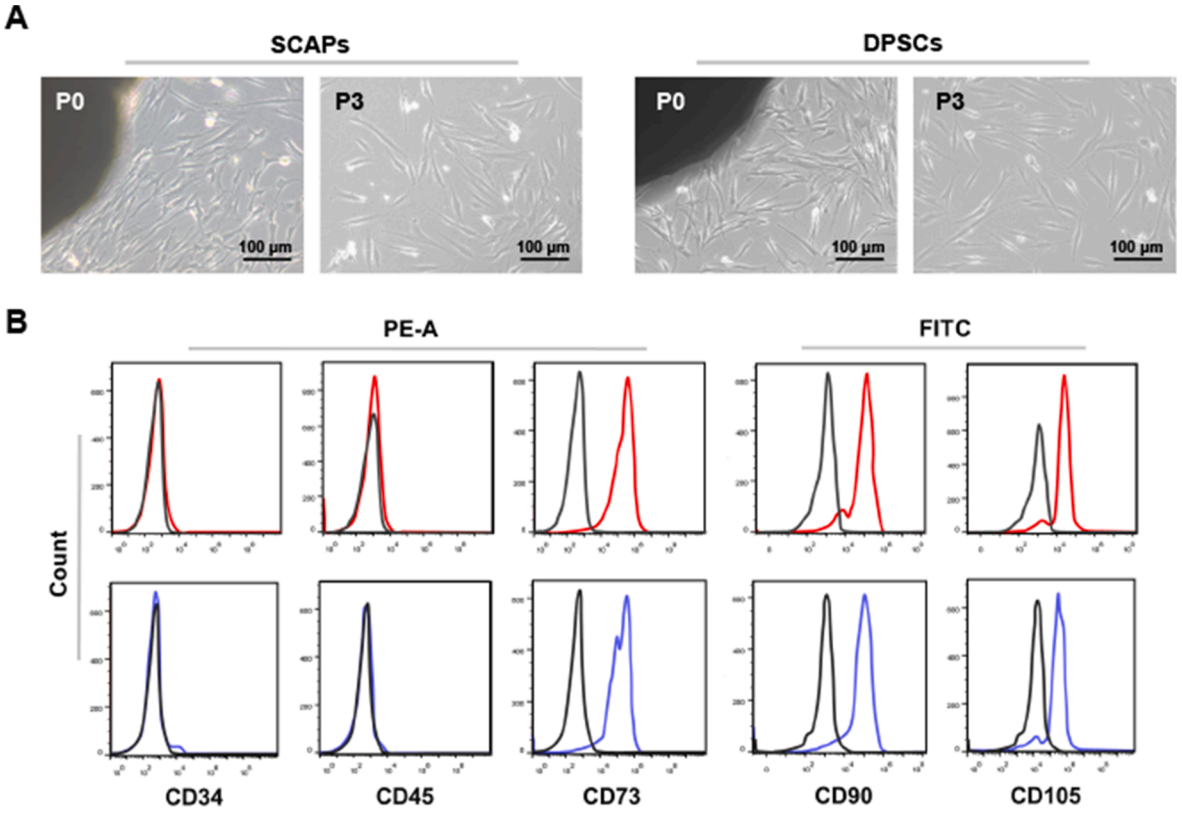

Figure 1 Isolation and identification of SCAPs and DPSCs. (A) The left shows the primary generation, and the right shows the P3 cells. (B) Identification of SCAPs and DPSCs by flow cytometry.

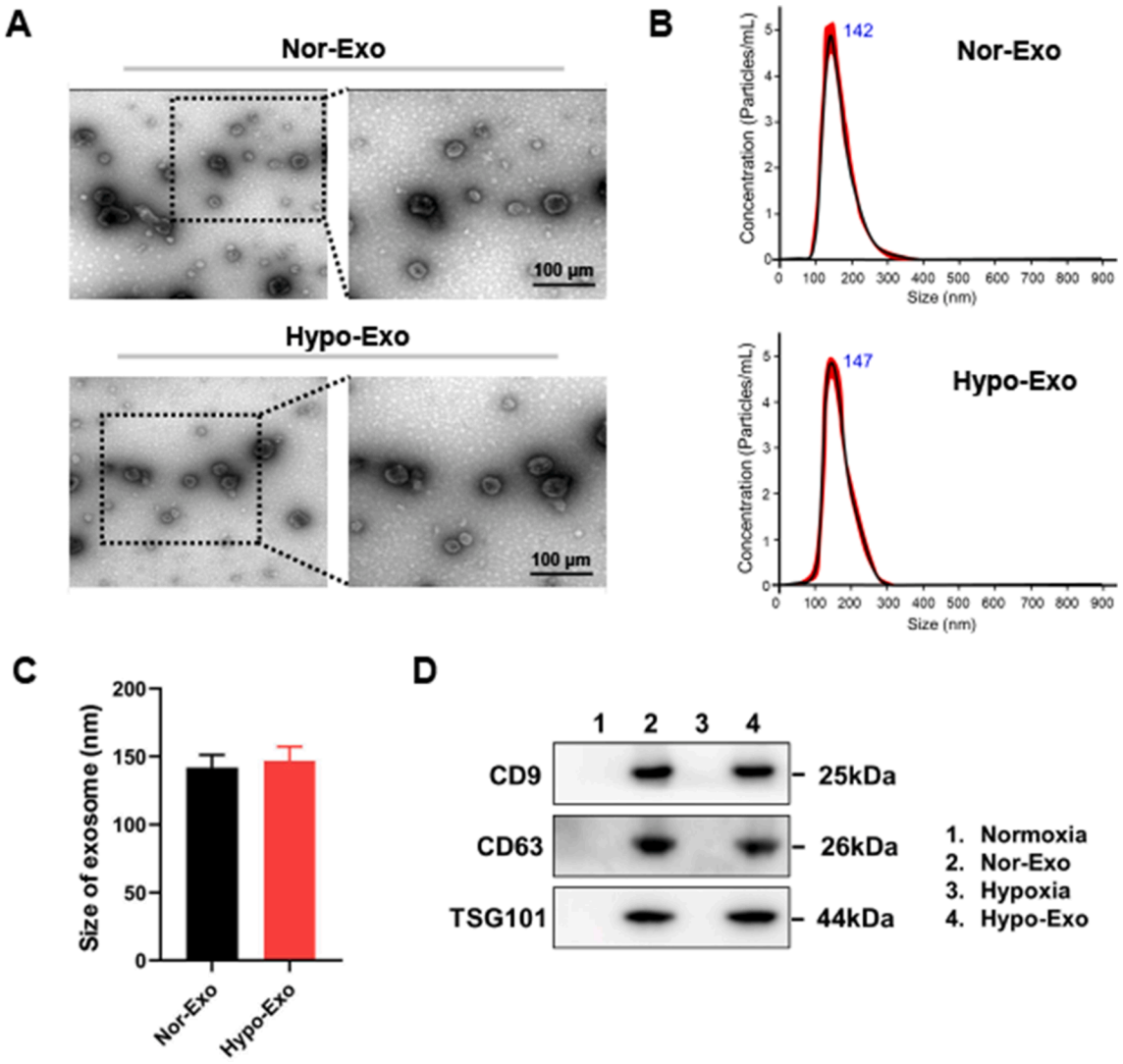

Figure 2 The morphology and particle size of exosomes secreted by SCAPs under hypoxia are not significantly different from those under normoxicity. (A) Observation of exosomes morphology in hypoxic and normoxic groups under transmission electron microscopy. (B, C) Detecting the distribution of particle size of two groups of extracellular vesicles using Nanoparticle tracking analysis. (D) Identification of two groups of exosomes by Western blot.

significant red fluorescence signals as early as 24 h post-co-culture (Fig. 3A). The mean fluorescence intensity between these two groups showed no significant difference, suggesting equivalent internalization efficiency of exosomes by DPSCs (Fig. 3B). Furthermore, we evaluated the karyotypes of DPSCs following co-culture with Nor-Exo and Hypo-Exo and found no detectable chromosomal aberrations in any of the tested groups (Fig. 3C). This observation aligns with previous reports indicating that exosomes derived from human SCAPs possess high biological safety.[21]

Scratch wound healing assays indicated that the healing rate of DPSCs treated with Hypo-Exo was significantly greater than that observed in the Nor-Exo group and the PBS group at the 12-h mark (Fig. 3D). Furthermore, transwell migration assays demonstrated that the number of transmembrane cells in the Hypo-Exo group exceeded that of the Nor-Exo group (Fig. 3E). These findings suggest that exosomes secreted by SCAPs can enhance the migratory capacity owef DPSCs, and that hypoxic treatment can further augment the ability of SCAP-derived exosomes to promote DPSC migration.

Regulatory effect of hypoxic SCAPs-derived exosomes on DPSCs odontogenic differentiation

RT-qPCR analysis revealed an upregulation in the expression levels of odontogenic differentiation-related genes, specifically *DSPP*, *ALP*, and *RUNX2*, in the Hypo-Exo group compared to the Nor-Exo group (Fig. 4A). This upregulation was further corroborated by Western blot analysis, which confirmed that exosomes derived from SCAPs enhance the expression of odontogenic differentiation markers in DPSCs following hypoxic treatment (Fig. 4B). These findings were consistent with the results obtained from ALP staining and

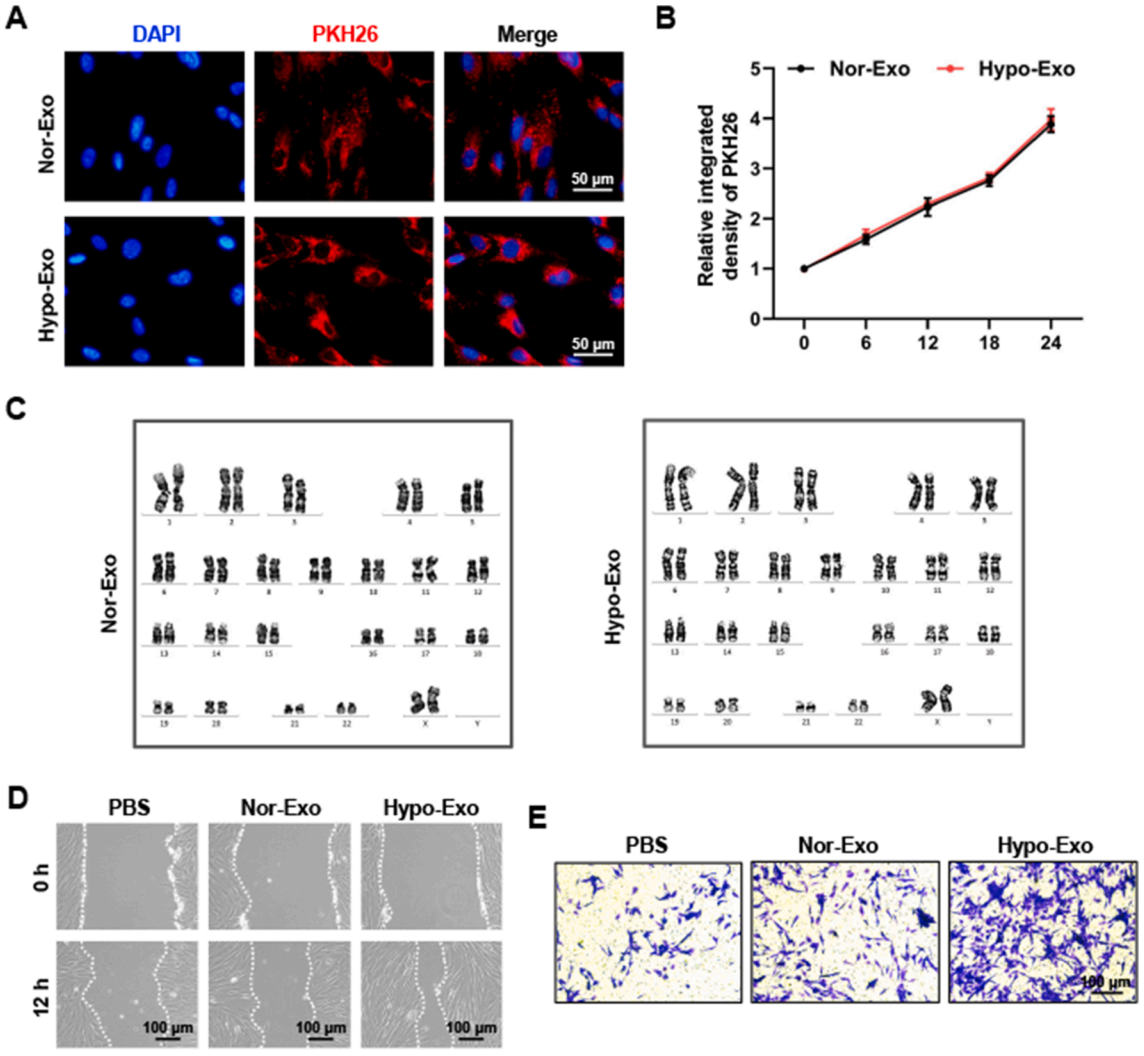

Figure 3 Exosomes from SCAPs in Hypo-Exo group promote DPSCs migration ability. (A, B) PKH26 fluorescence tracing experiments revealed no significant difference in the internalization efficiency of DPSCs between the two exosomes groups (n = 3). (C) Chromosomal stability was evaluated using karyotype analysis. (D) The effect of two exosomes groups on cell migration was assessed using scratch wound healing assays (n = 3). (E) The impact of the two exosomes groups on cell invasion was examined through transwell assays (n = 3).

alizarin red staining (Fig. 4C—F). Collectively, these data suggest that exosomes derived from hypoxia-treated SCAPs facilitate the odontogenic differentiation of DPSCs.

Hypoxic SCAPs-derived exosomes promote DPSCs migration and differentiation via mitochondrial fusion

Recent studies have demonstrated that mitochondrial fusion enhances cellular energy metabolism, thereby supplying essential energy for critical biological processes such as cell migration and directional differentiation.[22—24] Based on this, we hypothesized that mitochondrial fusion might mediate the regulatory effects of hypoxic SCAPs-derived exosomes on DPSCs migration and odontogenic differentiation (Fig. 5A). TEM observations of mitochondrial morphology in DPSCs stimulated by exosomes from both groups revealed that DPSCs co-cultured with hypoxic SCAP-derived exosomes exhibited significantly enlarged mitochondria compared to those co-cultured with normoxic SCAP-derived exosomes (Fig. 5B). This finding suggests that the co-culture of DPSCs with hypoxic SCAPs-derived exosomes promotes mitochondrial fusion in DPSCs. Furthermore, Western blot analysis confirmed the upregulated expression of mitochondrial fusion-related proteins in DPSCs treated with hypoxic exosomes (Fig. 5C).

Moreover, Seahorse assays and Mito-tracker red staining were conducted to examine the potential impact of mitochondrial fusion on the cellular energy metabolism of DPSCs. The results showed that DPSCs co-cultured with hypoxic exosomes exhibited enhanced energy metabolism compared to those co-cultured with normoxic exosomes (Fig. 5D—F). To elucidate the molecular mechanism by which mitochondrial fusion mediated cellular energy metabolism influences the migration and differentiation capabilities of DPSCs via Hypo-Exo, a rescue experiment

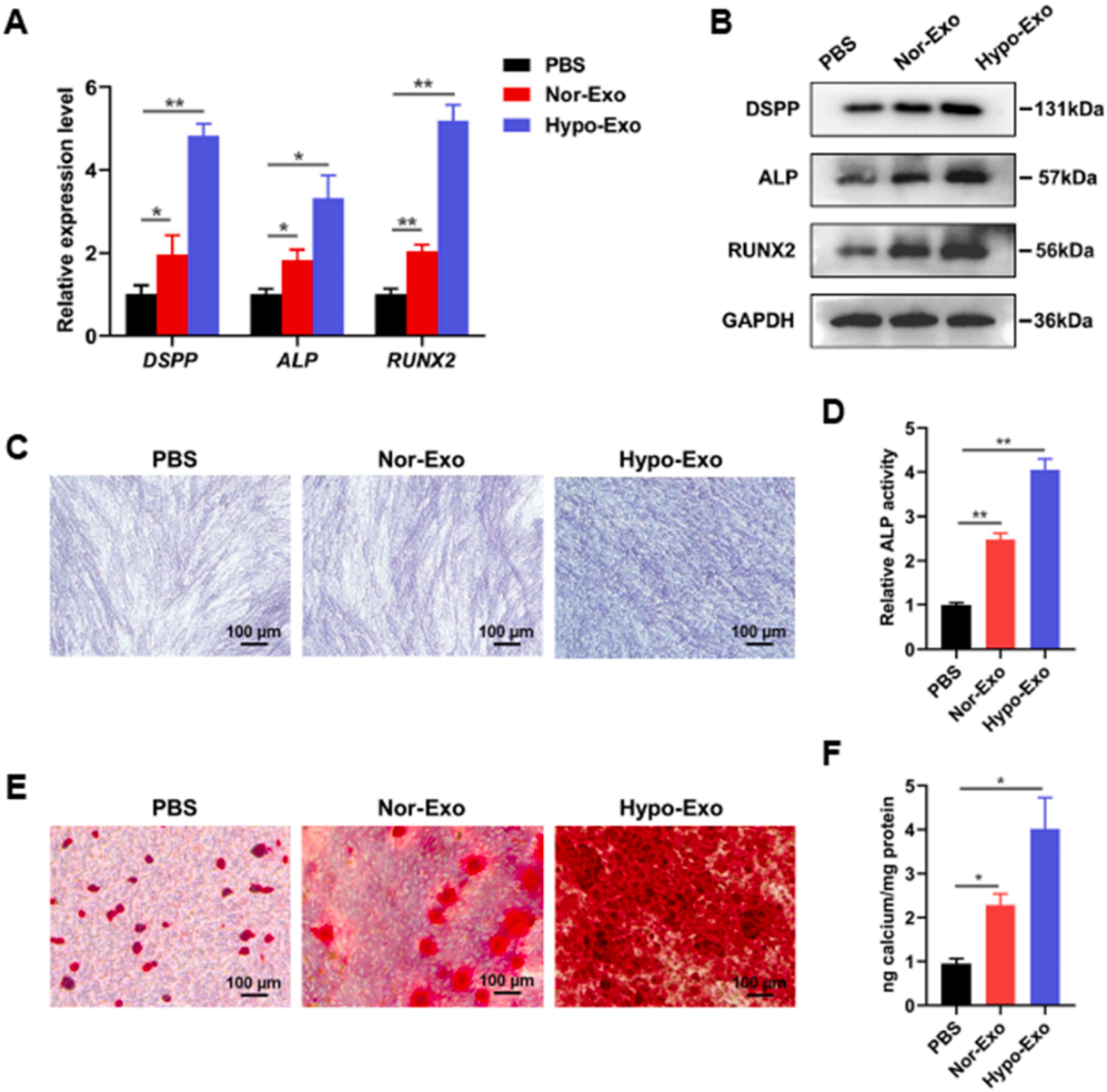

Figure 4 SCAPs-derived exosomes facilitate the odontogenic differentiation of DPSCs under hypoxic conditions. (A, B) The expression levels of odontogenic differentiation markers, including dentin sialophosphoprotein (DSPP), alkaline phosphatase (ALP), and runt-related transcription factor 2 (RUNX2) in DPSCs were assessed using RT-qPCR and Western blot analysis (n = 3). $^{*}P \leq 0.05$,$^{**}P \leq 0.01$ compared to control. (C—F) ALP staining, alizarin red staining, and subsequent quantitative analyses demonstrated that SCAPs-derived exosomes enhanced mineralization deposition in DPSCs under hypoxic conditions (n = 3). $^{*}P \leq 0.05$,$^{**}P \leq 0.01$ compared to control.

was designed. This involved the concurrent administration of rotenone to inhibit cellular energy metabolism. The Mito-tracker red staining results revealed that the addition of rotenone effectively inhibited the Hypo-Exo induced upregulation of energy metabolism in DPSCs (Fig. 6A and B). Scratch and migration assays further demonstrated that rotenone significantly counteracted the enhancement of DPSC migration ability induced by Hypo-Exo (Fig. 6C and D). Additionally, RT-qPCR and Western blot analyses confirmed that rotenone suppressed the Hypo-Exo-mediated upregulation of DSPP, ALP, and RUNX2 expression in DPSCs (Fig. 6E and F). Consistent conclusions were drawn from the results of alizarin red staining (Fig. 6G and H). Collectively, these data indicate that mitochondrial fusion-mediated energy metabolism is the key molecular mechanism underlying the regulatory effects of hypoxic SCAP-derived exosomes on DPSC biological properties.

Discussion

In recent years, there has been considerable scholarly interest in the role of exosomes in intercellular communication and tissue regeneration.[25,26] Empirical evidence

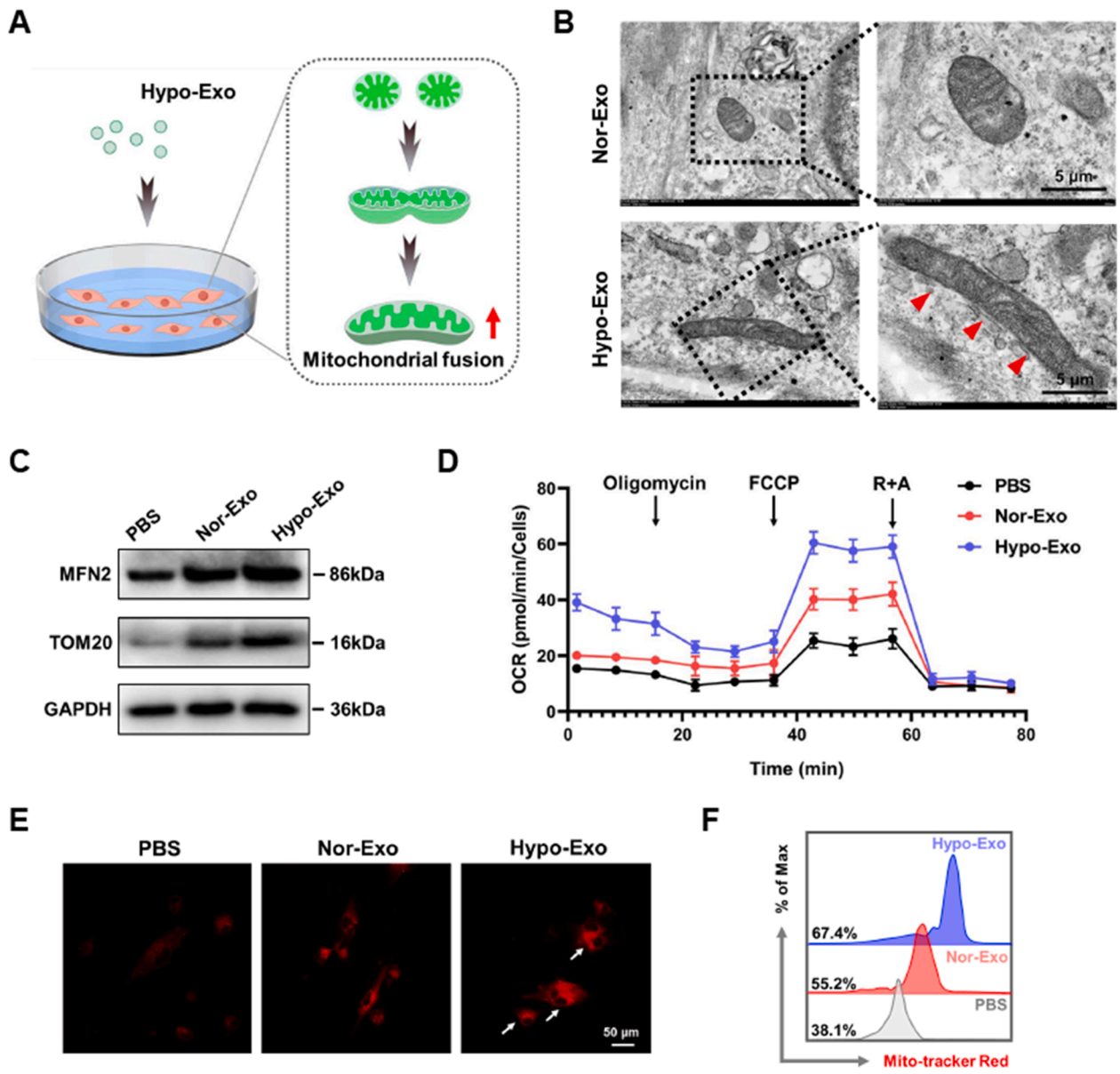

Figure 5 SCAPs-derived exosomes facilitate mitochondrial fusion in DPSCs under hypoxic conditions, thereby enhancing cellular energy metabolism. (A) A schematic diagram illustrates the mechanism through which exosomes from hypoxic SCAPs modulate the biological characteristics of DPSCs. (B) Transmission electron microscopy observed that compared to the Nor-Exo group, hypoxic SCAPs exosomes promoted mitochondrial fusion in DPSCs (n = 3). (C) Western blot assay was conducted to evaluate the expression levels of mitochondrial fusion related markers mitofusin 2 (MFN2) and translocator of the outer mitochondrial membrane 20 (TOM20) in DPSCs (n = 3). (D) The Seahorse experiment was employed to measure the cellular energy metabolism levels of DPSCs. (E, F) Mito-tracker red staining and subsequent flow cytometry analysis were used to evaluate the effect of exosomes derived from hypoxic SCAPs on the energy metabolism levels of DPSCs (n = 3).

indicates that exosomes derived from SCAPs under hypoxic conditions can significantly enhance angiogenesis, thereby aiding in the reconstruction of vascular networks during dental regeneration.[11] This study aims to systematically evaluate the effects of exosomes secreted by SCAPs in hypoxic microenvironments on the migration and odontogenic differentiation of DPSCs, with a particular focus on intercellular communication between SCAPs and DPSCs, positioning exosomes as the principal point of investigation.

Through scratch wound healing assays and transwell migration assays, we systematically assessed the regulatory effects of hypoxic SCAPs-derived exosomes on the migration of DPSCs. Additionally, we employed RT-qPCR, Western blot analysis, ALP and alizarin red staining to evaluate the odontogenic differentiation potential. The findings revealed that DPSCs treated with hypoxic exosomes demonstrated significantly enhanced migration capacity and commitment differentiation. Previous studies have

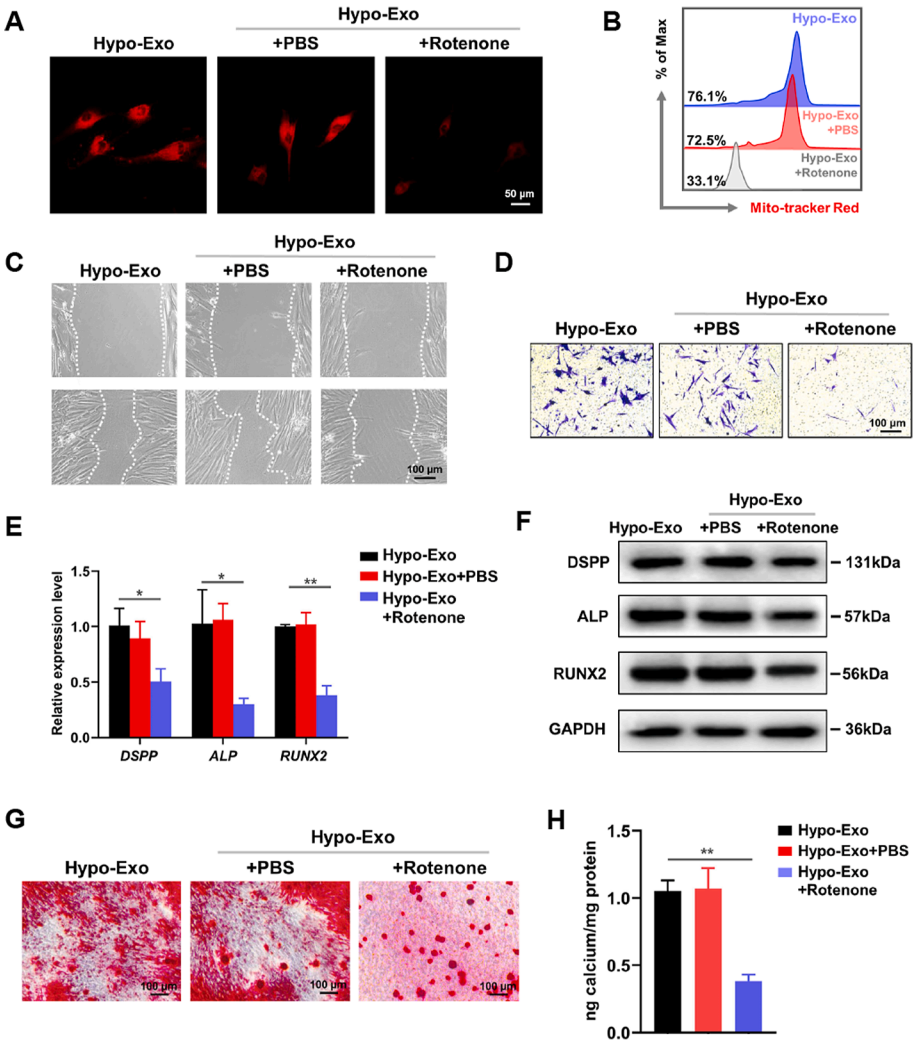

Figure 6 The rescue experiment demonstrates that exosomes derived from hypoxic SCAPs regulate the biological characteristics of DPSCs through alterations in cellular energy metabolism. (A, B) The addition of rotenone inhibited the upregulation of energy metabolism levels in DPSCs induced by hypoxic SCAPs exosomes (n = 3). (C, D) Detecting the effect of rotenone on the upregulation of energy metabolism of hypoxic SCAPs exosomes in DPSCs via scratch and transwell assays (n = 3). (E, F) RT-qPCR and Western blot were used to detect the effect of rotenone on odontogenic differentiation markers in DPSCs (n = 3). $^{*}P \leq 0.05$,$^{**}P \leq 0.01$ compared to control. (G, H) The effect of rotenone on the mineralization of DPSCs facilitated by exosomes from hypoxic SCAPs was assessed using alizarin red staining and corresponding quantitative experiments (n = 3). $^{**}P \leq 0.01$ compared to control.

suggested that the alterations in the biological characteristics of recipient cells induced by hypoxic exosomes may be attributed to the hypoxic microenvironment's influence on exosome size or the internalization efficiency of the recipient cells.[27–29] In this study, we conducted a comprehensive analysis of the morphology and particle size distribution of exosomes derived from SCAPs cultured under normoxic and hypoxic conditions. Our findings indicated no significant differences in either the morphology or particle size between the two groups of exosomes. Furthermore, PKH26 fluorescent tracing demonstrated that there was no significant difference in the uptake capacity of DPSCs for these two types of exosomes. This suggests that exosomes derived from hypoxic SCAPs may transfer specific bioactive molecules, such as odontogenic microRNAs or signaling proteins, to influence DPSC migration and odontogenic differentiation. In other words, hypoxia appears to primarily modulate the biological effects of SCAPs-derived exosomes by altering their cargo composition rather than their physical properties.

Mitochondria, often referred to as the "powerhouses" of the cell, are crucial for maintaining normal physiological functions and engaging in essential biological processes.[30] Recent studies have demonstrated that mitochondrial fusion enhances cellular energy metabolism, thereby supplying the necessary energy for vital biological activities.[31,32] Transmission electron microscopy has shown that the average mitochondrial length in DPSCs treated with hypoxic exosomes is greater than that observed in the control group. This increase in length is accompanied by a significant upregulation of fusion-related proteins, including mitofusin 2 (MFN2) and translocator of the outer mitochondrial

membrane 20 (TOM20).[33,34] This morphological alteration is closely associated with functional improvements, as evidenced by Seahorse assay and Mito-tracker red staining, which indicate a marked increase in basal OCR and a notable enhancement in ATP production efficiency. These findings suggest that mitochondrial energy metabolism plays a significant role in facilitating the pro-migratory and odontogenic effects of hypoxic SCAPs' exosomes.

To further substantiate the role of mitochondrial fusion in the regulatory effects of hypoxic SCAP-derived exosomes on the migration and commitment differentiation of DPSCs, a series of rescue experiments were conducted. Following treatment with rotenone, an inhibitor of mitochondrial energy metabolism, a marked decrease was observed in the capacity of hypoxic SCAP-derived exosomes to enhance DPSC migration and directional differentiation.[35,36] These results corroborate the hypothesis that mitochondrial fusion-mediated energy metabolism constitutes the principal molecular mechanism driving the regulatory effects of hypoxic SCAP-derived exosomes on DPSC biological properties. This underscores the pivotal role of mitochondrial dynamics balance in this process. The findings provide novel experimental evidence supporting the theoretical framework of "mitochondrial dynamics-metabolic reprogramming-cell fate determination".

In conclusion, this study provides novel insights into the intercellular communication between SCAPs and DPSCs, while also establishing an experimental framework for the development of exosome-based dental regeneration strategies. Furthermore, comprehensive investigations into mitochondrial fusion are anticipated to offer theoretical foundations for uncovering new mechanisms that govern cell migration and directional differentiation. Future research should aim to elucidate the specific bioactive molecules present in hypoxic SCAP-derived exosomes and their mechanisms of action, as well as explore the relationships between mitochondrial fusion and other cellular biological processes. Such endeavors are expected to significantly advance the field of oral tissue engineering.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgements

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