Primary Cilia Mediate Wnt5a/ β -catenin Signaling to Regulate Adipogenic Differentiation of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells Following Calcium Induction

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Abstract

BACKGROUND: Regeneration of soft tissue defects is essential for adipose tissue pathologies and disease, trauma, or injury-induced damage. Here, we show that umbilical cord blood-derived mesenchymal stem cells could potentially be tailored and used for the reconstruction of specific damaged sites. Adipogenesis can be exploited in soft tissue reconstruction. Also, primary cilia play a role in the control of adipogenesis.

METHODS: The adipogenic differentiation capacity of mesenchymal stem cells (MSCs) was shown to influence ciliogenesis. MSCs transfected with intraflagellar transport 88 (IFT88) small interfering RNA (siRNA), which blocks the assembly and maintenance of cilia, were examined to confirm the relationship between adipogenesis and ciliogenesis. Also, 1,2-Bis(2-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM), calcium chelator, inhibited the ciliogenesis of MSCs in adipogenic differentiation.

RESULTS: IFT88-knockdown led to decreased cilia formation and limitation of cilia elongation in adipogenesis. Additionally, intracellular calcium triggered cilia formation in MSCs adipogenesis. Interestingly, intracellular calcium cannot overcome the inhibition of adipogenesis caused by low numbers of cilia in MSCs.

CONCLUSION: Our data suggested that ciliogenesis was negatively regulated by Wnt5a/ β -catenin signaling during adipogenesis. Thus, we suggest that calcium induction triggers adipogenesis and ciliogenesis.

Keywords Adipogenesis · Mesenchymal stem cell · Cilia · Calcium · Wnt

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1 Introduction

Regenerative medicine holds great promise in repairing damaged tissues and organs and restoring their functionality by stimulating the body's regenerative capacity. Particularly, adipose tissue regeneration is a growing field that addresses current clinical needs for restoration or establishment of normal function in various adipose tissue pathologies and defects. In this regard, various cell sources have been utilized in adipose tissue regeneration strategies [1, 2]. Human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) are a promising cell source for tissue regeneration, which can reconstruct the damage at the site of external wounds or surgical resections. Recently, stem cell transplantations were performed for soft tissue

regeneration in conditions such as burns or skin aging [3, 4]. Besides their capacity to differentiate into adipocytes, osteocytes, chondrocytes, neuronal cells, etc., MSCs have several other advantages such as easier isolation, high proliferation rate, and immunomodulation [5, 6]. Therefore, MSCs have a marked potential for a broad range of therapeutic applications.

The primary cilia, present in most of the cells in the organism, acts as a sensory organelle that transfers information from the environment to the cell interior. The cilia are crucial for regulating important cellular processes, including the cell cycle, cytoskeletal organization, intraflagellar transport, and signaling pathways such as the hedgehog (Hh), notch, and canonical and non-canonical Wnt/planar cell polarity (PCP) pathways [7]. There have been several studies which showed that primary cilia play a role in the control of adipogenesis. The primary cilia were shown to modulate size modification during adipocyte differentiation of human adipose stromal cells [8]. Moreover, the primary cilia controlled the adipose progenitors [9].

Our previous research suggested that calcium regulated the adipogenic differentiation of MSCs by negatively regulating Wnt5a/ β -catenin signaling [10]. Calcium has a role in the physiological mediator for activating various intracellular signaling pathways [11, 12]. Calcium is also known to act as a booster in the early stages of adipocyte differentiation via cAMP induction, which increases the gene expression of peroxisome proliferator-activated receptor gamma (PPAR γ) [13, 14]. Among the Wnt ligands expressed on MSCs, Wnt3 and Wnt10 in particular, regulate the β -catenin pathway to activate the differentiation of preadipocytes into adipocytes [15, 16]. Also, canonical Wnt pathway, leading the suppressed adipogenesis, is triggered by intracellular calcium release [17, 18]. Therefore, it is important to clarify the role of calcium in MSCs adipogenesis and ciliogenesis.

Here, we have investigated whether the cilia were involved in MSCs adipogenesis in the presence of calcium. In addition, the signaling pathways related to adipogenesis and ciliogenesis were elucidated for the first time. Together, the primary cilia may promote adipogenesis by negatively regulating Wnt5a/ β -catenin signaling via intracellular calcium. Our findings suggest that MSCs are an effective candidate for the reconstruction of large soft tissue defects with respect to the improvement of primary cilia function in adipogenesis.

2 Materials and methods

2.1 Cell preparation and culture conditions

Human UCBs from umbilical veins were collected and isolated within 24 h after neonatal delivery with informed

maternal consent. UCB-MSCs were separated from mononuclear cells (MNCs) with Ficoll-Hypaque solution ($d = 1.077 \text{ g/cm}^3$, GE Healthcare, Uppsala, Sweden). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (MEDIPOST Co. Ltd., P01-201601-31-003). Collected MSCs were washed and cultured in minimum essential medium alpha (Gibco/Invitrogen, Carlsbad, Grand Island, NY, USA) supplemented with 10% Fetal bovine serum (FBS; Gibco) at 37 °C in a 5% CO₂ incubator [19]. The culture medium was changed twice a week. MSCs (passage 6) used for experiments were obtained from six different donors.

2.2 Adipogenic differentiation

To induce adipogenic differentiation, MSCs were cultured with an adipogenic medium consisting of high glucose Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% FBS, 0.5% gentamicin (Gibco), 0.5 mM 3-isobutyl-1-methylxanthine, 1 mM dexamethasone, 0.2 mM indomethacin, and 10 mM h-insulin (Sigma-Aldrich Co., St. Louis, MO, USA) for 2 weeks. Assessment of adipocyte formation was based on the number of accumulated lipid vacuoles which were determined by staining with oil red O solution. Cells were treated with a calcium chloride solution (1.8 mM, Sigma) for 10 days. Subsequently, cells were incubated with 1,2-Bis(2-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM, 5 μ M, Calbiochem, La Jolla, CA, USA), an intracellular calcium chelator, for 1 h in serum-free media before adipogenic induction. MSCs were further incubated for 24 h, with or without 1 μ M Smo agonist (SAG, TOCRIS, Bristol, UK). Depending on their adipogenic differentiation potential, MSCs were distinguished into MSCs-H (M-H) and MSCs-L (M-L). Each group consisted of MSCs from three donors.

2.3 Western blotting

Cell extracts were prepared in RIPA buffer (ThermoFisher Scientific, Waltham, MA, USA). Approximately 15 μ g of protein extracts were separated by SDS–polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. Antibodies against Wnt5a (1:500, Abcam, Cambridge, UK), β -catenin (1:100, Abcam), PPAR γ (1:1000, Santa Cruz Biotechnology, Dallas, TX, USA), leptin (1:1000, Thermo Fisher Scientific), intraflagellar transport 88 (1:1000, proteintech, Rosemont, IL, USA), and GAPDH (1:2000, Novus, Centennial, CO, USA) were used and detected using an Goat anti-Rabbit IgG(H + L)-HRP secondary antibody (1:2000, GenDEPOT, Barker,

TX, USA). The chemiluminescent signals were analyzed using the ChemiDocTM MP Imaging System (Bio-Rad, Hercules, CA, USA).

2.4 Quantitative real-time polymerase chain reaction (qPCR) and small interfering RNA (siRNA)

The mRNA expression of the PPAR γ (NM_138712.3) and CEBP α (NM_001287424.2) genes was analyzed and quantified by qPCR using a LightCyclerTM 480 system (Roche, Mannheim, Germany) and normalized to the GAPDH mRNA (NM_002046.7). TaqMan probes were designed using the Universal ProbeLibrary Assay Design Center (Roche). Cells were transfected with IFT88 small interfering RNAs (siRNAs) and Scrambled siRNA (50 nM, Dharmacon, Lafayette, CO, USA) for 24 h using the DharmaFECT reagent according to the manufacturer's recommendations. The sequences used were: IFT88 siRNA (GAGAAUUAUAUGAUCGUGA, AGGCAA AUGGAACGUGAAA, AGGAAGUGCUAGCGGUGAU, AGUAAAGGUGAACGACUA A); scramble siRNA (UGGUUUACAUGUUGUGUGA, UGGUUUACAUGUUUCUGA, UGGUUUACAUGUUUCCUA).

2.5 Immunofluorescent staining

To detect the cilia, cells were seeded on two-chamber slides. After washing with phosphate buffered saline, cells were fixed with 4% PFA and then blocked with 0.1% Triton X-100. Cells were then incubated with a primary antibody (mouse monoclonal anti acetylated tubulin, 1:1000, Sigma) followed by an appropriate secondary antibody (anti-mouse Alexa Fluor[®] 488, 1:300, Jackson ImmunoResearch Europe Ltd., Newmarket, UK). IFT88 on the fixed cells was detected using a rabbit polyclonal primary antibody (1:50, Proteintech) followed by an anti-rabbit Cy3-conjugated secondary antibody (1:300, Jackson). To detect adipogenic differentiation, PPAR γ protein levels were detected using a rabbit polyclonal PPAR γ primary antibody (1:100, Novus) followed by an anti-rabbit Cy3-conjugated secondary antibody (1:300, Jackson). Fluorescent images were acquired and analyzed using the LSM 800 confocal microscope (Zeiss, Oberkochen, Germany).

2.6 Statistical analysis

All data were presented as mean $\pm$ standard error. Statistical analysis was performed with a one-way analysis of variance followed by Tukey's HSD test using GraphPad

Prism 6 (San Diego, CA, USA). Data were considered statistically significant if $p < 0.05$.

3 Results

3.1 Primary cilia elongation occurs in response to adipogenic media

To evaluate the ciliation of MSCs in adipogenic induction media, 6 lots of MSCs were selected and cultured in adipogenic induction media for 2 weeks. Based on the potential of adipogenic differentiation, MSCs from the 6 different MSC lots were divided into two groups: M-L (MSC1-MSC3) and M-H (MSC4-MSC6). The differentiation of adipogenic potential between M-L and M-H was shown with oil red O staining (Supplemental Fig. 1A). Several ciliated cells were present in M-H following adipogenic differentiation. However, less ciliated cells were observed in M-L (Fig. 1A). Following adipogenic induction, the M-H group showed an increase in the number of ciliated cells, as well as cilia elongation (Fig. 1B, C). There were significant differences regarding ciliation between the two groups even before undergoing adipogenic differentiation (Supplemental Fig. 1B-D). As such, to determine the function of primary cilia during adipogenic differentiation, we treated cells in the M-L group with SAG (cilia enhancer, 1 μ M) for 24 h prior to induction of adipogenic differentiation. After treatment with a cilia enhancer, the number of lipid vesicles in MSCs cultured in adipogenic induction medium was increased (Fig. 1D). The increased number of cilia induced by SAG treatment was shown to regulate the expression of the adipogenic gene marker PPAR γ , and cilia marker acetylated α -tubulin, during adipogenic differentiation (Fig. 1E). These results suggest that cilia elongation depends on the adipogenic differentiation potential of MSCs.

3.2 Primary cilia are necessary for adipogenic differentiation of MSCs

To identify the role of cilia in adipogenesis, we silenced IFT88, a marker of cilia retraction, along with acetylated tubulin in MSCs. We transfected MSCs with 50 nM IFT88 siRNA or a scrambled control siRNA for 24 h (Supplemental Fig. 2). There was no significant change in the expression of genes associated with adipogenesis, such as PPAR γ and CEBP α , in IFT88 silenced M-L cells. qRT-PCR analysis of IFT88 silenced M-H cells revealed that IFT88 is required for induction of PPAR γ and CEBP α , suggesting that cilia are critical in the early stages of adipogenic differentiation (Fig. 2A, B). We confirmed the knockdown of IFT88 in M-H cells via immunofluorescent

Fig. 1 Ciliated MSCs depend on the adipogenic differentiation potential. **A** The ciliation of MSCs varies depending on adipogenic differentiation potential. MSC-L (M-L) group showed low adipogenic differentiation capacity. MSC-H (M-H) group showed high adipogenic differentiation capacity. Immuno-stained MSCs with anti-acetylated α -tubulin was used to identify MSCs cilia following adipogenic differentiation for 2 weeks. Scale bar = 20 μ m. **B** Following adipogenic induction, the percentage of ciliated cells stained by acetylated α -tubulin increased significantly. **C** The cilia length was analyzed via imaging. While culturing in adipogenic-specific

medium, the length of cilia of MSCs was increased. **D** Oil red O staining quantified adipogenic differentiation by staining the lipid vesicles. Neutral lipids were increased in MSC1 (M-L) cells grown in adipogenic-specific medium and treated with 1 μ M SAG (cilia enhancer). Scale bar = 200 μ m. **E** Following SAG treatment for 24 h, the expression of the adipogenic marker PPAR γ was increased in MSC1 cells, indicating that ciliation is associated with the adipogenic differentiation of MSCs. Ciliation of MSC1 cells was also increased following SAG treatment. Scale bar = 20 μ m. Data are presented as mean $\pm$ SD, n = 3 per group. ** p < 0.01

staining. The identified role of cilia in regulating adipogenesis was also confirmed via double immunofluorescent staining against acetylated α -tubulin and PPAR γ antibodies (Fig. 2C, D). Our results revealed a suppressed expression

of acetylated α -tubulin and PPAR γ during adipogenesis in IFT88 siRNA transfected M-H cells. Thus, removal of IFT88 affected adipogenesis, indicating that adipogenesis is dependent on the cilia.

Fig. 2 Suppression of cilia formation impaired the adipogenic differentiation of MSCs. **A**, **B** IFT88-knockdown by IFT88 siRNA (50 nM) in MSCs was analyzed by qRT-PCR. Inhibition of adipogenic differentiation by blocking primary cilia formation was determined. Gene expression of adipogenic markers, PPAR γ and CEBP α , was significantly decreased following IFT88-knockdown in

the M-H group. **C** Immunofluorescence microscopy (IF) analysis in the M-H group showed that IFT88-knockdown shortened cilia formation during adipogenic differentiation for 2 weeks. **D** IFT88 siRNA transfected MSCs showed a decrease in PPAR γ gene expression. Scale bar = 20 μ m. Data are presented as mean $\pm$ SD, $n = 3$ per group. * $p < 0.05$

3.3 Ciliation increase during adipogenic differentiation is associated with increased calcium levels

We previously showed the association between intracellular calcium levels and adipogenic differentiation [10]. The increase of extracellular calcium levels leads to an increase in calcium influx through the calcium channel. To assess the effect of calcium or BAPTA-AM on adipogenic differentiation, cells were treated with 1.8 mM of calcium chloride for 10 days (over two passages). In the presence of intracellular calcium during adipogenesis, MSCs showed an increase in the number of lipid vacuoles as quantified by oil red O staining (Fig. 3A). Gene expression of both PPAR γ and CEBP α was increased in M-H cells with increased intracellular calcium prior to adipogenic differentiation (Fig. 3B, C). MSCs from both groups (M-L and M-H), at which they had high levels of intracellular

calcium, were cultured in adipogenic induction media for 2 weeks. To clarify the effect of calcium on adipogenic differentiation, BAPTA-AM, a cell-permeant intracellular calcium chelator, was used to pre-treat cells for 1 h prior to induction of adipogenic differentiation. The number of ciliated cells in the M-H group was decreased, as quantified by immunofluorescent staining (Fig. 3D). Notably, increased intracellular calcium levels led to a significant increase in the percentage of ciliated cells in both M-L and M-H groups (Fig. 3E). However, the length of the cilia was not affected by intracellular calcium levels. Likewise, no significant difference in cilia length was observed between cells treated with calcium and BAPTA-AM (Fig. 3F). Together, these results indicate that intracellular calcium affects ciliation during adipogenic differentiation.

Fig. 3 Calcium triggered an increase of ciliated MSCs during adipogenic differentiation. **A** Oil red O staining showed the difference of adipogenic differentiation potential between the M-L and M-H groups in the presence of calcium. Neutral lipids were detected after 2 weeks of differentiation with/without intracellular calcium. Scale bar is 200 μ m. **B, C** qRT-PCR analysis of calcium treated M-L and M-H cells was used to determine the expression of early adipocyte genes PPAR γ and CEBP α and was normalized to the GAPDH mRNA. Data are presented as mean $\pm$ SD, $n = 3$ per group. **D** Primary cilia, which appeared in the M-L and M-H cells, were identified using anti-acetylated α -tubulin. Cells were treated for 1 h with BAPTA-AM, a calcium chelator, in serum-free media to block

the effects of calcium on ciliation, followed by adipogenic differentiation. Scale bar = 20 μ m. **E** The percentage of ciliated cells in the M-L and M-H groups was quantified and analyzed by staining cells with α -tubulin. Cells were treated with calcium and BAPTA-AM to determine the effects of calcium on adipogenesis and ciliation after 2 weeks. **F** Cilia length was significantly higher in MSCs treated with calcium or adipogenic induction medium in the M-H group. The percentage of ciliated cells and cilia measurement was made from 5 preparations in each group and approximately 10 cilia were randomly selected in each preparation. The graph shows individual values of cilia length from measurements made in cells treated with calcium or adipogenic induction medium. $^{**}p < 0.01$; $^{*}p < 0.05$

3.4 Intracellular calcium promotes increased numbers of ciliated cells during adipogenesis

In order to confirm that increased calcium levels during adipogenic differentiation enhance the number of ciliated UCB-MSCs, we transfected MSCs with IFT88 siRNA and scramble siRNA after treatment with 1.8 mM of calcium chloride for 10 days (over two passages), followed by adipogenic differentiation for 2 weeks. IFT88-knockdown cells showed a low level of adipogenic differentiation with the increase of intracellular calcium levels (Fig. 4A). These results were consistent with those obtained by real-time

PCR. Knockdown of the cilia-associated protein IFT88 downregulated the expression of adipogenic genes PPAR γ and CEBP α in the presence of intracellular calcium (Fig. 4B, C). MSCs expression of IFT88 was determined by immunostaining after intracellular calcium dependent-adipogenic differentiation. To assess cilia co-localization, cells in the M-H group were co-immunostained for α -tubulin. IFT88 colocalized to the base and tip of the ciliary axoneme with the increase of intracellular calcium levels during adipogenic differentiation (Fig. 4D). This indicated that the function of IFT88 may be important in the early signals of adipogenic lineage specification in MSCs.

Fig. 4 Intracellular calcium triggered ciliation and adipogenesis by negatively regulating Wnt5a/ β -catenin signaling. **A** Oil red O staining was used to determine neutral lipids at 2 weeks. IFT88 siRNA (50 nM) transfected MSCs were cultured with adipogenic induction medium with/without intracellular calcium. **B, C** Adipogenic markers, PPAR γ and CEBP α , were analyzed in the presence of intracellular calcium. **D** Immunofluorescent staining was used to show cilia

formation induced by intracellular calcium levels. While examining the role of calcium in adipogenic differentiation, primary cilia formation was determined using α -tubulin and IFT88 antibodies. **E** M-H cells were transfected with IFT88 siRNA for 24 h. Wnt5a, β -Catenin, PPAR γ , CEBP α , Leptin, and IFT88 antibodies were used to analyze the IFT88-knockdown in MSCs. Data are presented as mean $\pm$ SD, $n = 3$ per group. $^{**}p < 0.01$

IFT88-knockdown M-H cells were cultured in adipogenic induction media for 1 week to further examine the relationship between ciliation and Wnt5a/ β -catenin signaling during adipogenic differentiation. Following transfection with IFT88 in M-H cells for 24 h, Wnt5a and β -catenin expression were significantly increased. In contrast, PPAR γ and leptin expression was decreased. Thus, our results showed that Wnt5a/ β -catenin signaling was negatively regulated by adipogenesis and ciliation (Fig. 4E). Our previous research demonstrated that intracellular calcium triggered adipogenesis by negatively regulating Wnt5a/ β -catenin signaling [10]. Furthermore, we examined the Wnt5a/ β -catenin signaling pathway as an important regulator of MSCs ciliogenesis and adipogenesis. Thus, we conclude that the Wnt5a/ β -catenin signaling pathway is negatively regulated by increased intracellular calcium

levels, thereby suppressing MSCs adipogenesis and ciliogenesis.

4 Discussion

This study has demonstrated that primary cilia are prevalent in MSCs and that cilia appear to play a critical role in mediating the adipogenic differentiation of MSCs. Additionally, we have found that the presence of calcium during adipogenic differentiation enhanced both the number of ciliated cells and the length of the cilia. With the induction of intracellular calcium, the number of ciliated cells was increased but the length of the cilia was not affected. To demonstrate the role of cilia in MSCs adipogenesis, we created IFT88-knockdown UCB-MSCs, which showed that the disruption of cilia structure led to a reduction in

adipogenesis. Overall, the increase of intracellular calcium level in MSCs accelerated the reduction of Wnt5a/ β -catenin, which triggered adipogenesis and ciliogenesis.

Adipogenesis is important for the regeneration of large soft tissue defects [20, 21]. A better understating of adipogenesis in soft tissue reconstruction, as well as cellular physiology and factors influencing adipogenesis, will help to develop strategies to make adipose tissue more effective for repairing large defects caused by various pathologies and trauma. However, little is known about the relationship between adipogenesis and ciliogenesis. Human bone marrow-derived MSCs (BMSCs), adipose-derived MSCs (ASCs), and pluripotent cells were shown to be suitable for clinical adipose tissue engineering [22]. As such, the use of UCB-MSCs for adipose tissue engineering has been prevalent in recent years [3, 4]. High yields and proliferative rates, as well as low immunogenicity, make UCB-MSCs a superior clinical cell source. Consequently, the therapeutic potential of UCB-MSCs is enormous. Therefore, it is critical to understand the molecular mechanism involved in the adipogenic differentiation of UCB-MSCs. We tested the six MSCs populations isolated from different donors to determine their adipogenic differentiation capacity. The M-H group, which had high adipogenic differentiation potential, showed an increase in both the number of ciliated cells and cilia length. Also, the M-L group, showing the lower adipogenic differentiation potential, showed the high levels of ciliate cells and cilia length in adipogenic differentiation. To determine the interaction between ciliogenesis and adipogenesis, MSCs were treated with SAG, cilia enhancer, during adipogenesis. With the enhancement of cilia formation, the increase of adipogenic differentiation gene marker, PPAR γ , was observed. As such, our results indicate that ciliogenesis is dependent upon the adipogenic differentiation potential of UCB-MSCs. Cilia formation depends on the adipogenic differentiation potential of mesenchymal stem cells.

Ciliopathy is associated with a genetic disorder of the cellular cilia or the cilia anchoring structures, the basal bodies, or of ciliary function. Ciliopathies, such as Bardet-Biedl (BBS) and Alstrom syndromes (ALMS) present with obesity as a clinical feature [23–25]. However, it is unclear how disrupted ciliary function leads to obesity. IFT88 helps mediate intraflagellar transport, an important process essential for the assembly and maintenance of both primary and motile cilia, as well as flagella. IFT88 signal outlines the original shape of the cilia, while acetylated tubulin marks the shrinking microtubules of the retreating cilia. The acetylation of the K40 residue in α -tubulin is a hallmark of stable microtubules and is also used to gauge cilia retraction in tandem with IFT88 [26]. In this study, we silenced IFT88 expression by siRNA transfection in UCB-MSCs to determine the role of cilia formation and

retraction in adipogenesis. Overall, we found that adipogenesis is associated with cilia formation.

In our previous research, we suggested that calcium plays a key role in the early stages of adipogenesis in UCB-MSCs. Also, our previous research showed that intracellular calcium levels of ASCs and BMSCs were higher than those of UCB-MSCs, having lower levels of adipogenesis than other sources of stem cells [10]. This can be explained by the association between intracellular calcium concentration and senescence [27, 28]. UCB-MSCs can have a low senescence with a lower intracellular calcium level, providing the clue for low adipogenic differentiation potential. Here, the induction of calcium triggered cilia formation and adipogenic differentiation. The expression of adipogenic gene markers, PPAR γ and CEBP α , was significantly increased in the M-H group following calcium induction. Intracellular calcium enhanced cilia elongation, which was confirmed by immunofluorescent staining of acetylated α -tubulin. On the other hand, BAPTA-AM, a calcium chelator, led to a decrease in the number of ciliated cells. Previous reports supported the critical role of primary cilia in multiple differentiation pathways, including osteogenic and adipogenic differentiation. IFT88 siRNA transfection in UCB-MSCs led to inhibition of primary cilia formation, as well as lower differentiation capacity. Therefore, the role of primary cilia is guiding lineage commitment and maintaining the phenotype of differentiated cells [26, 29].

To examine the role of Wnt signaling in adipogenesis and ciliogenesis, we tested whether Wnt5a and β -catenin expression in the M-H and M-L groups was depended on the adipogenic differentiation potential. Herein, we show that Wnt signaling, likely mediated by Wnt5a, plays a role in adipogenesis. These results were explained by previous research, which Wnt10b/ β -catenin inhibits the gene expression of PPAR γ , interrupting adipocyte formation [15]. Moreover, our data showed that following IFT88 siRNA transfection, Wnt5a and β -catenin expression was significantly increased in UCB-MSCs. Similarly, it has been shown that various stem and progenitor cells alter the length of their primary cilia. Previous reports suggested that Wnt signaling in mouse embryonic fibroblasts and MSCs was associated with the function and structure of primary cilia which affected their differentiation potential [30, 31]. Notably, MSCs cultured on nanotopographical grooved surfaces exhibited enhanced ciliogenesis, which in turn, inhibited canonical Wnt activity. Furthermore, knockdown of IFT88 led to a decrease in the length of cilia in ASCs [32].

In conclusion, primary cilia mediate adipogenesis by negatively regulating Wnt5a/ β -catenin signaling. Adipogenesis and ciliogenesis were enhanced in the presence of intracellular calcium. As such, we demonstrated that

calcium is a key regulator of both adipogenesis and ciliogenesis. Therefore, primary cilia expressed on UCB-MSCs may play a role in inducing adipogenic lineage-specific differentiation to restore defected soft tissues.

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Authors' contribution SU and JHJ supervised the study and wrote the manuscript; YKB and GK performed the experiments; JHK and MK contributed to analyze the results; SJC and WO organized the study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement The study protocol was approved by the institutional review board of the institutional and/or national research committee (MEDIPST Co. Ltd., P01-201601-31-003). Informed consent was confirmed by the IRB.

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