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Simvastatin modulates osteogenic differentiation in Stem Cells isolated from Apical Papilla

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Abstract

Background Simvastatin modulates numerous stem cell functions, including stemness maintenance and differentiation. The present study aimed to explore the effect of simvastatin on the osteogenic differentiation of Stem Cells isolated from Apical Papilla (SCAPs) in vitro.

Methods Cells were isolated from apical papilla, and mesenchymal stem cell features were characterised. Cells were treated with various concentrations of simvastatin (100-1,000 nM). The mRNA expression profile of simvastatin-treated SCAPs was examined using RNA sequencing technique. The osteogenic differentiation abilities were assessed. Alkaline phosphatase activity was determined. The mineralisation was visualised using Alizarin Red S and Von Kossa staining. The osteogenic marker gene expression was determined using a quantitative polymerase chain reaction.

Results RNA sequencing data demonstrated that simvastatin upregulated genes enriched in those pathways involving osteogenic differentiation, including the TGF-β signalling pathway, FoxO signalling pathway, and MAPK signalling pathway, while the downregulated genes were involved in pathways related to cell proliferation and apoptosis, for example, DNA replication, cell cycle, and p53 signalling pathway. Simvastatin promoted mineral deposition in a dose-dependent manner, corresponding with the upregulation of osteogenic marker genes namely *OSX*, *DMP1*, *DSPP*, and *OCN*. Pretreatment with TGF-β receptor inhibitor, SB431542, resulted in a moderately attenuated effect on simvastatin-induced mineralisation and osteogenic marker gene expression.

Conclusions Simvastatin enhances osteogenic differentiation in SCAPs, potentially via TGF-β signalling, implicating its potential role as an adjunctive molecule in dental pulp healing and regeneration in vital pulp treatment approaches.

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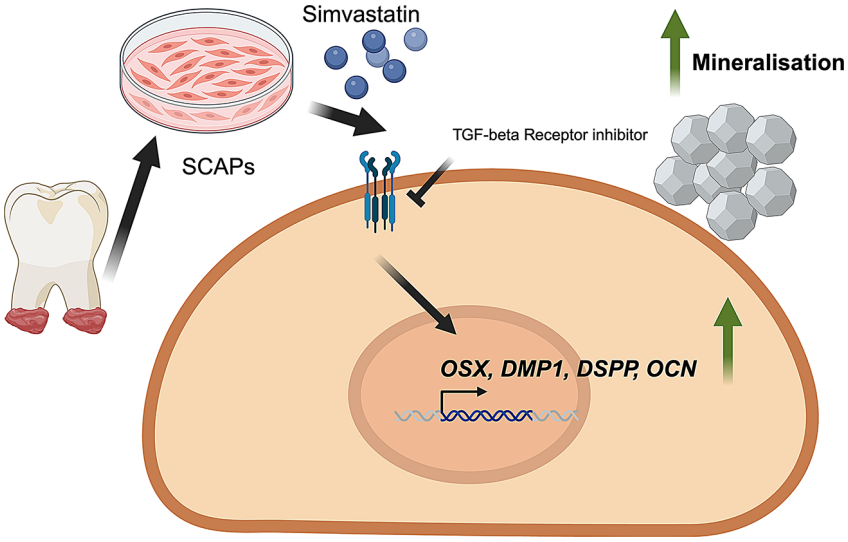
Graphical Abstract



Keywords Stem cells from apical papilla, Simvastatin, Cell viability, Cell proliferation, Cell migration

Introduction

Simvastatin, a commonly used cholesterol-lowering medication, has demonstrated its potential in modulating stem cell functions, including stemness maintenance and differentiation. In canine bone marrow-derived mesenchymal stem cells, simvastatin enhanced proliferation and inhibited apoptosis [1]. Low-dose simvastatin upregulated mRNA expression of pluripotent stem cell markers, *Rex1* and *Oct4* [1]. On the contrary, a high concentration of simvastatin attenuated this stemness modulation [1]. Simvastatin decreased intracellular lipid accumulation and adipogenic marker gene expression in murine marrow stromal cells [2]. Simvastatin promoted the proliferation of human dental pulp stem cells (hDPSCs) by activating the PI3K/AKT pathway [3]. Simvastatin also exhibited a role in inflammation. In this respect, Simvastatin treatment rescued LPS-induced inflammatory gene expression in hDPSCs via the regulation of ERK1/2 and p38 pathways [4].

Additionally, simvastatin is known for promoting bone regeneration and osteogenic differentiation [5]. Recent studies have explored the use of simvastatin in enhancing the osteogenic potential of stem cells. In this regard, simvastatin-loaded collagen sponge facilitates bone tunnel defect healing in rat femur, demonstrating the significant reduction of defect area and a marked increase of bone mineral density [6]. The femur defects treated with simvastatin-loaded sponge demonstrated a higher load per displacement ratio compared to the control group, which implicated the improvement of mechanical properties of simvastatin-induced regenerated bone [6]. Simvastatin induced mRNA expression of *RUNX2*, *OPG*, *OPN*, and *OSX* in mesenchymal stem cells (MSCs) [7]. This osteogenic inductive property is potentially regulated through the BMP2/Smad pathway [7].

Stem cells isolated from apical papillae (SCAPs) are one of the stem cells in the oral cavity that are derived from MSCs [8] and a promising source for dental tissue regeneration, especially considered as the crucial cell source in regenerative endodontic procedures. SCAPs transplantation with platelet-rich plasma in the lumen of the tooth root segment resulted in the formation of dentin-pulp-like structures in the subcutaneous implantation model, indicating the vital role of SCAPs in this regenerative approach [9]. In these regenerative processes, the bioactive molecules are beneficial in promoting cell migration, proliferation, and odonto/osteogenic differentiation of SCAPs. Hence, the present study aimed to investigate the effect of simvastatin on the osteogenic differentiation of SCAPs in vitro and the potential regulatory mechanism was also explored.

Materials and methods

Cell isolation and culture

The immature third molars were obtained from patients scheduled for tooth extraction at the Faculty of Dentistry, Chulalongkorn University, according to their treatment plan, with written informed consent obtained. The study protocol was approved by the Human Research Ethical Committee, Faculty of Dentistry, Chulalongkorn University (approval No. 062/2022). Apical papilla tissues were collected for cell explantation [10]. Cells were cultured in Dulbecco-modified Eagle medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 10% fetal

bovine serum (FBS, Gibco), 2 mM L-glutamine (GlutaMAXTM, Gibco), 100 µg/mL streptomycin, and 100 U/ml penicillin (Sigma-Aldrich, USA) at 37 ° C in a humidified atmosphere with 5% CO_2. Stem cell surface markers and differentiation ability towards osteogenic and adipogenic lineages were performed following established protocols [10, 11]. Cells were treated with simvastatin (cat. No. 000008154, Sigma-Aldrich) at a concentration range from 100 nM to 1000 nM, based on previous studies demonstrating its effects on SCAPs [12]. For inhibition experiment, cells were pretreated with TGF-β receptor inhibitor, SB431542, (8 µM, cat. no. 1614, Sigma–Aldrich, USA) 30 min prior to simvastatin exposure. DMSO was employed as the vehicle control. For osteogenic differentiation, cells were seeded in 24-well plates at 5×10^4 cells/well with growth medium. After 24 h, cells were cultured in an osteogenic induction medium supplemented with simvastatin. The medium was changed every two days.

Mineral deposition assay

Cells were fixed with cold methanol for 10 min and stained with 1% w/v Alizarin Red S (Sigma Aldrich) solution at room temperature for 3 min. Samples were washed with deionised water between each step. Calcium deposition was quantified by destaining with 10% cetylpyridinium chloride monohydrate (Sigma Aldrich) solution. The absorbance of the solution was measured using a microplate reader (Biotek ELX800, USA) at 570 nm. The absorbance values were normalised to each sample's control culture in osteogenic differentiation medium without simvastatin. For Von Kossa staining, samples were fixed with 4% formaldehyde in PBS and incubated with 5% silver nitrate in sterile deionised water under UV light for 15 min at room temperature.

Alkaline phosphatase enzymatic assay

The cells were fixed with a 4% paraformaldehyde solution for 10 min and then incubated with BCIP/NBT tablets (Roche) for 30 min in the dark at room temperature. The ALP-stained cells were examined under the microscope.

RNA sequencing

Cells were treated with simvastatin (100 nM) for 24 h. RNA isolation was executed utilising the RNeasy kit (Qiagen, USA), followed by validation of RNA quality through an Agilent 2100 BioAnalyzer (Agilent Technologies, USA) and NanoDrop (Thermo Fisher Scientific Inc.). Library construction was performed with the NEBNext® Ultra™ RNA Library Prep Kit for Illumina®, and subsequent validation and quantification were conducted using an Agilent 2100 BioAnalyzer and Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Sequencing was executed on the NextSeq 500 platform, with reads processed and filtered via FastQC and the FastQ Toolkit, and data alignment to the human reference genome (GRCh38) was performed using the software HISAT2 (v2.0.1) [13, 14]. After the clean data were aligned to the reference genome using Hisat2, HTSeq (v0.6.1) was employed with the reference gene file to estimate gene and isoform expression levels from the paired-end clean data [15]. Differential expression analysis was facilitated through the DESeq2 Bioconductor package, and bioinformatics analyses were conducted using WebGestalt [16] and KEGG for pathway enrichment assessment. A heatmap of significantly altered gene expression was generated using Heatmapper software [17]. The sequencing data were submitted to NCBI's Gene Expression Omnibus (GSE286540).

Quantitative Polymerase Chain Reaction (qPCR)

Total RNA was extracted using TRIzol reagent (RiboEx solution, Cat. No. 301–001, GeneAll, South Korea) following the manufacturer's protocol. Subsequently, 2 µg of RNA was reverse-transcribed to synthesise first-strand complementary DNA (cDNA) using a kit from Gibco BRL (Rockville, MD, USA). The cDNA was amplified in a reaction volume of 20 µL, containing 2.5 mmol/L magnesium dichloride, 1.25 U of Ex Taq Polymerase (Bioneer, Daejeon, Korea), and 1 mmol/L specific primers. Thermocycling conditions included an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 40 s, annealing at 55 °C for 40 s, and extension at 72 °C for 90 s, with a final extension at 72 °C for 10 min. The relative gene expression was calculated using the comparative Ct ($2^{-\Delta\Delta Ct}$) method [18]. The *GAPDH* mRNA was used as the internal control for normalisation. The primer sequences used for PCR are listed in Supplementary Table 1.

Statistical analyses

Experiments were performed with biological replications from different donors ($n = 3$–12). Data were presented as mean±standard deviation. The statistical analysis was performed with Prism 10 software (GraphPad Software, USA). The Mann-Whitney U test was utilised for the comparison of two independent groups, whereas the Kruskal-Wallis test, accompanied by pairwise analyses, was employed for the comparison of three or more groups, with a p-value threshold of less than 0.05, denoting statistical significance.

Results

Gene expression profile of simvastatin-treated scaps

The isolated cells expressed CD44, CD90, and CD105 but not CD45 (Fig. 1A). Marked mineral deposition and intracellular lipid accumulation were observed when cells were maintained in osteogenic and adipogenic induction conditions (Fig. 1B-E), confirming mesenchymal stem cell characters. After SCAPs were exposed to simvastatin. A total of 463 upregulated and 559 downregulated genes

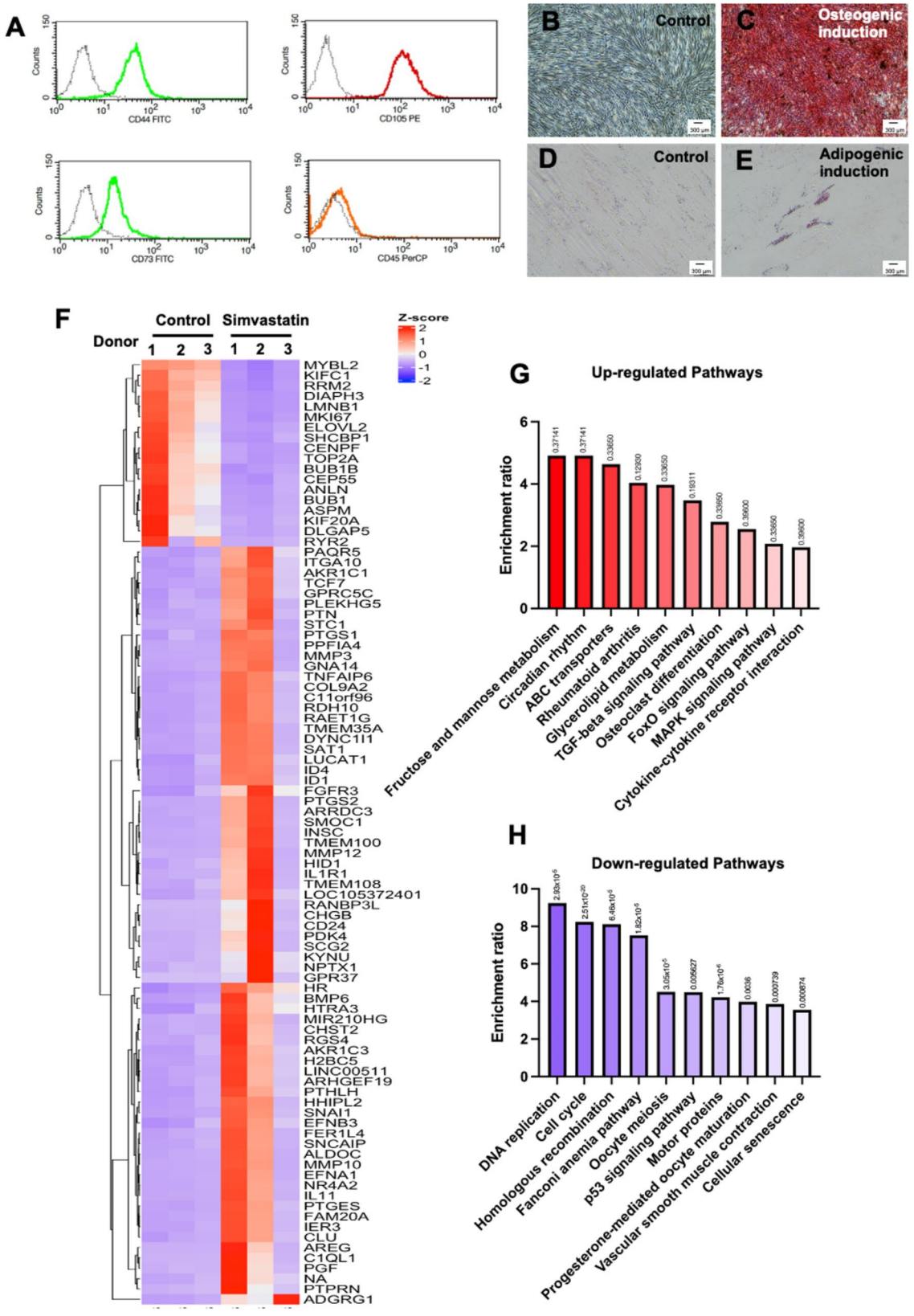


Fig. 1 (See legend on next page.)

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Fig. 1 *Gene expression profile of simvastatin-treated SCAPs.* Surface marker protein (CD44, CD90, CD105, and CD45) was analysed by flow cytometry (**A**). The mineral deposition was examined using Alizarin Red S staining at day 14 after cells were maintained in an osteogenic induction medium (**C**). Cells in a growth medium were used as the control (**B**). The intracellular lipid accumulation was examined using Oil Red O staining on day 16 after cells had been maintained in an adipogenic induction medium (**D**). Cells in a growth medium were used as the control (**E**). Heatmap demonstrated the differentially expressed genes in simvastatin-treated SCAPs compared to the control (**F**). Graphs illustrated the KEGG-enriched pathways for upregulated and downregulated genes, respectively (**G** and **H**)

were significantly differentially expressed. The heatmap of 91 differentially expressed genes (base mean > 150, $\log_2$foldchange>|2.5|) is shown in Fig. 1F. Pathway enrichment highlighted that the upregulated genes were enriched in those pathways involving in osteogenic differentiation, including the TGF-β signalling pathway, FoxO signalling pathway, and MAPK signalling pathway (Fig. 1G). Downregulated genes were involved in pathways related to cell proliferation and apoptosis for example DNA replication, cell cycle, and p53 signalling pathway (Fig. 1H). Those differentially expressed genes are illustrated as heatmaps in Fig. 2A-I. *BMP6* and *MKI67* were selected to validate gene expression determined by RNA sequencing (Fig. 2J and K).

Simvastatin promoted osteogenic differentiation in SCAPs

ALP enzymatic activity was decreased when cells were exposed to simvastatin in a dose-dependent manner at day 7 (Fig. 3A). On the contrary, simvastatin treatment promoted mineral deposition at day 14 after osteogenic induction in a dose-dependent manner, demonstrated by Alizarin Red S and Von Kossa staining (Fig. 3A and B). A significant increase in mineral deposition was noted in those cells treated with 1,000 nM simvastatin, corresponding with a marked increase of *OSX*, *DMP1*, *DSPP*, and *OCN* (Fig. 3F-L). The reduction of *RUNX2* and *ALP* mRNA expression was noted on simvastatin treatment (1,000 nm), while *COL1* and *OPN* mRNA levels did not alter in simvastatin-treated conditions (Fig. 3F-L).

According to RNA sequencing analysis, the TGF-β signalling pathway exhibited significant enrichment among the upregulated genes. To assess the participation of this pathway, cells underwent pretreatment with a TGF-β receptor inhibitor (SB431542) for 30 min before exposure to simvastatin (1,000 nM). The findings indicated that SB431542 moderately reduced simvastatin-induced mineral deposition by SCAPs (Fig. 4A). Furthermore, SB431542 also slightly diminished the expression of *OSX*, *DMP1*, and *DSPP* mRNA induced by simvastatin while not affecting *OCN* mRNA expression (Fig. 4B-E).

Discussion

The present study described the effects of simvastatin on SCAPs. Simvastatin-treated cells upregulated genes enriched in the osteogenic differentiation pathway (TGF-β signalling pathway, FoxO signalling pathway, and MAPK signalling pathway), while the downregulated genes were enriched in those pathways related to cell proliferation and cell apoptosis similar to those observed in hDPSCs and bone marrow-derived mesenchymal stem cells. Supplemented simvastatin in osteogenic induction medium enhanced osteogenic differentiation, confirmed by mineralisation and osteogenic marker gene expression. Further, the simvastatin-induced osteogenic differentiation potentially occurred via the TGF-β pathway.

SCAPs osteogenic differentiation cultures exhibited ALP activity as early as one week after induction, maintaining high levels even after three weeks, despite extensive mineral deposition partially hindering ALP substrate penetration [19]. In this study, ALP staining intensity decreased over time, in contrast to the increasing mineral deposition observed with Alizarin Red S and Von Kossa staining. This decline in ALP staining is likely due to SCAPs transitioning to late-stage differentiation before day 7, as ALP is an early osteogenic marker. With simvastatin accelerating differentiation, ALP activity declined more rapidly, reflecting a faster progression to late-stage osteogenesis.

The observed increase in *OSX*, *DMP1*, *DSPP*, *and OCN*, along with a reduction in *RUNX2* and *ALP*, indicates a shift to late-stage osteogenic differentiation, aligning with mineral deposition assay results. The decline in *RUNX2* and *ALP* suggests the completion of early differentiation [20], while the continued expression of *OSX*, *DMP1*, *DSPP*, and *OCN* corresponds with ongoing matrix mineralisation. Our findings align with a study reporting that despite the fading expression of *RUNX2*, *OSX* remains highly expressed in odontoblasts and dental pulp cells during late tooth development, particularly in *DSPP*-rich odontoblasts, suggesting a distinct regulatory role [21]. This consistency supports the notion that *OSX* may function independently of *RUNX2* during late-stage differentiation, reinforcing potential differences in the regulation of osteoblast and odontoblast maturation [22].

The present study demonstrated that the gene expression profile of downregulated genes enriched in numerous pathways in association with cell cycle and cell apoptosis. The present study also demonstrated the differentially expressed genes in p53 pathways in simvastatin-treated cells, implicating a role in cell apoptosis. Previous study illustrated the significant increase of subG0 population in simvastatin-treated SCAPs [12]. In hDPSCs, simvastatin alone did not influence cell apoptosis, but co-stimulation with LPS dramatically increased

apoptotic cells [4]. Further, several cell proliferation pathways were enriched, including cell cycle, DNA replication, and homologous recombination. Previous report demonstrated that simvastatin (500-1,000 nM) reduced cell number and colony forming unit in a dose-dependent manner in SCAPs [12]. A study in human and canine dental pulp stem cells demonstrated that simvastatin (1 μM) inhibited cell proliferation, but a higher dose at 5–10 μM led to cytotoxicity [23, 24]. On the contrary, simvastatin (1 μM) markedly increased the percentage of cell proliferation of hDPSCs [3]. Similarly, simvastatin at a dose of 2–10 μg/mL promoted cell proliferation, while at a dose of 15–20 μg/mL showed the inhibition effects of cell proliferation [4]. The discrepancies of simvastatin influences on cell proliferation could be due to different cell types, concentrations, and culture medium.

Some dead cells were observed when SCAPs were cultured in osteogenic differentiation medium containing simvastatin for 14 days. Two potential mechanisms were considered: simvastatin-induced excessive calcification causing cellular stress and subsequent cell death, or direct simvastatin-induced cell death with calcium deposition as a secondary effect. The upregulation of osteogenic gene markers supports the first mechanism, suggesting that simvastatin accelerates osteogenic differentiation beyond the cells' capacity to maintain homeostasis, leading to cell death. These findings align with the upregulated pathways such as TGF-β, FoxO, and MAPK signaling.

In the present study, the upregulated genes in simvastatin-treated SCAPs were enriched in pathways potentially involved in the modulation of osteogenic differentiation, including the TGF-β signalling pathway, the FoxO signalling pathway, and the MAPK signalling pathway. The participation of FoxO signalling in simvastatin treatment has been reported in several studies [25, 26]. However, the exact mechanism of simvastatin in the control of FoxO family, especially in the control of osteogenic differentiation, is yet unknown. *FoxO3* has been shown to regulate osteogenic differentiation. FoxO3 overexpression promoted osteogenesis but inhibited adipogenesis in murine femurs [27]. Overexpression *FoxO3* in murine bone marrow-derived mesenchymal stem cells enhanced *Runx2* expression in those cells from aged mice, while FoxO3 knockdown showed the opposite effects [27]. In human periodontal ligament cells, *FoxO1* overexpression dramatically increased alkaline phosphatase activity, mineralisation, and *RUNX2* and *COL1A1* expression, indicating its function in the control of osteogenic differentiation [28]. This regulation is modulated via PI3K/AKT/mTOR pathway [28]. Interestingly, FoxO1 agonist injection at the periodontitis inductive site in rat models resulted in the attenuation of alveolar bone loss [29]. However, the bidirectional effect of FoxO on osteogenic regulation by interaction with Runx2 and β-catenin in MSCs was also proposed [30]. In SCAPs, there was no study related to FoxO in controlling cell behaviours. However, Foxc2, another subfamily of Fox family, upregulated *ALP*, *DSPP*, *DMP1*, and *OCN* mRNA expression and the effects on osteogenic differentiation in SCAPs were enhanced with co-overexpressed Foxc2 with BMP2 [31]. Additionally, the PI3K/AKT pathway has been implicated in the regulation of osteogenic differentiation, as its inhibition by FGF2 pretreatment has been shown to enhance the osteo/odontogenic potential of SCAPs [32]. Wnt signaling is also involved, as its inhibition by SFRP2 in SCAPs reduces β-catenin activity, enhances osteogenic marker expression, and promotes osteo/dentinogenesis [33]. Similarly, WIF1, a canonical Wnt/β-catenin inhibitor, enhanced dentinogenic differentiation in SCAPs by activating the transcription factor OSX, further supporting the role of Wnt inhibition in promoting SCAP differentiation [34]. The participation of these pathways on simvastatin-regulated osteogenic differentiation in SCAPs required further investigation.

Numerous evidence indicate the participation of the MAPK pathway in the regulation of osteogenic differentiation in SCAPs. Previous reports indicated that mineral trioxide aggregate (MTA) promoted osteogenic-related gene expression in SCAPs via MAPK pathways. In this regard, MTA increased pERK and p-p38 protein expression [35]. Further, the p38 and ERK inhibitors attenuated the MTA-induced *RUNX2*, *DSPP*, *BSP*, and *OCN* in SCAPs [35]. MAPK signalling is also involved in enhancing osteogenic differentiation by both biological and mechanical stimulation, i.e. parathyroid hormone and compressive mechanical force [36, 37]. In hDPSCs, simvastatin attenuated LPS-induced inflammatory cytokine and vascular endothelial growth factor expression, potentially via the modulation of ERK1/2 and p38 pathway [4]. Similarly, Tideglusib-doped nanoparticles (TDg-NPs) have been found to enhance osteogenic differentiation and mineralisation of hDPSCs despite inflammatory challenges, primarily through modulation of Wnt/β-catenin and MAPK signaling pathways [38].

It has been reported that biomaterials incorporated with TGF-β1 enhanced odontogenic differentiation in SCAPs as shown by the significant increase of and *DMP1* mRNA and protein expression, corresponding with the marked increase of mineralisation observed by Alizarin Red S staining [39, 40]. On the other hand, TGF-β1 treatment increased pSmad3/Smad3 and pERK1/2/ERK1/2 ratio and significantly inhibited alkaline phosphatase enzymatic activity and mineralisation in SCAPs [41]. The addition of TGF-β receptor inhibitor, SB431542, together with forskolin, dramatically enhanced alkaline phosphatase enzymatic activity and mineralisation [41]. Our present study illustrated that simvastatin enhanced

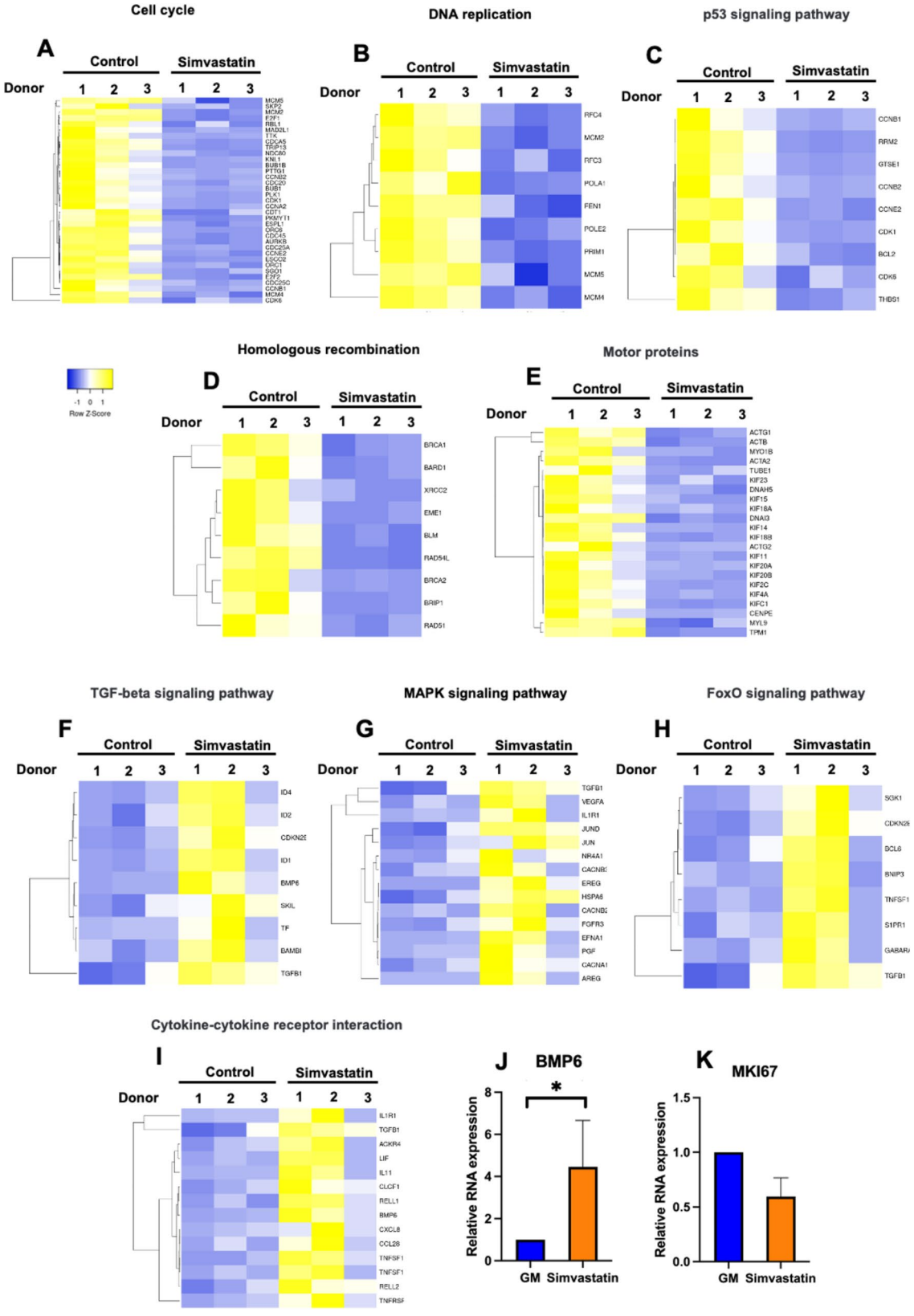

Fig. 2 *Gene expression profile of simvastatin-treated SCAPs.* Heatmaps of differentially expressed genes were illustrated for enriched pathways, including cell cycle (**A**), DNA replication (**B**), p53 signalling pathway (**C**), Homologous recombination (**D**), motor proteins (**E**), TGF- β signalling pathway (**F**), MAPK signalling pathway, FoxO signalling pathway (**H**), and cytokine-cytokine receptor interaction (**I**). *BMP6* (**J**) and *MKI67* (**K**) mRNA expression were examined using quantitative polymerase chain reaction to validate RNA sequencing data

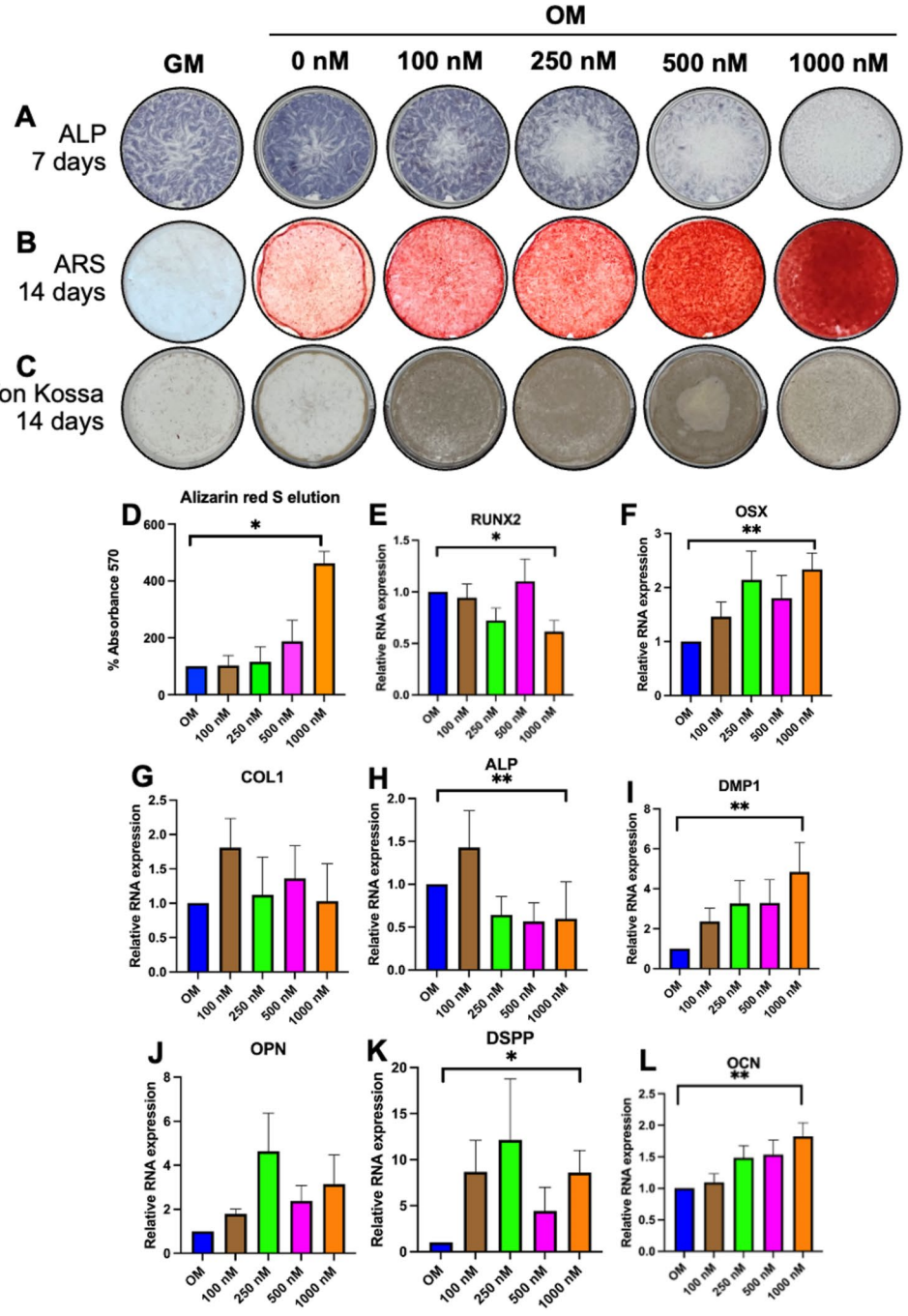

Fig. 3 *Simvastatin promoted osteogenic differentiation in SCAPs.* Cells were treated with simvastatin at the concentration range from 100-1,000 nM. (**A**) Alkaline phosphatase enzymatic activity was determined at day 7. (**B**) Alizarin Red S staining and (**C**) Von Kossa staining were utilised for detecting mineral deposition at day 14. (**D**) Alizarin Red S was solubilised and the percentage of absorbance at 570 nm was demonstrated. (**E-L**) The mRNA levels of osteogenic marker genes were determined using a quantitative polymerase chain reaction on day 7. Bars indicate a statistically significant difference. GM; growth medium, OM; osteogenic induction medium, $*p < 0.05$, $**p < 0.01$

mineral deposition and osteogenic marker gene expression in SCAPs, and this effect was moderately rescued by the addition of SB431542, implicating the crucial role of TGF-β1 signalling in simvastatin-promoted osteogenic differentiation in SCAPs.

As it was proved in other cell types, simvastatin promoted odontogenic and osteogenic differentiation. It has been proposed as a candidate bioactive molecule for enhancing hard tissue formation. The exemplification for this is that the formation of a periodontal ligament stem cell sheet on a polycaprolactone membrane loaded with

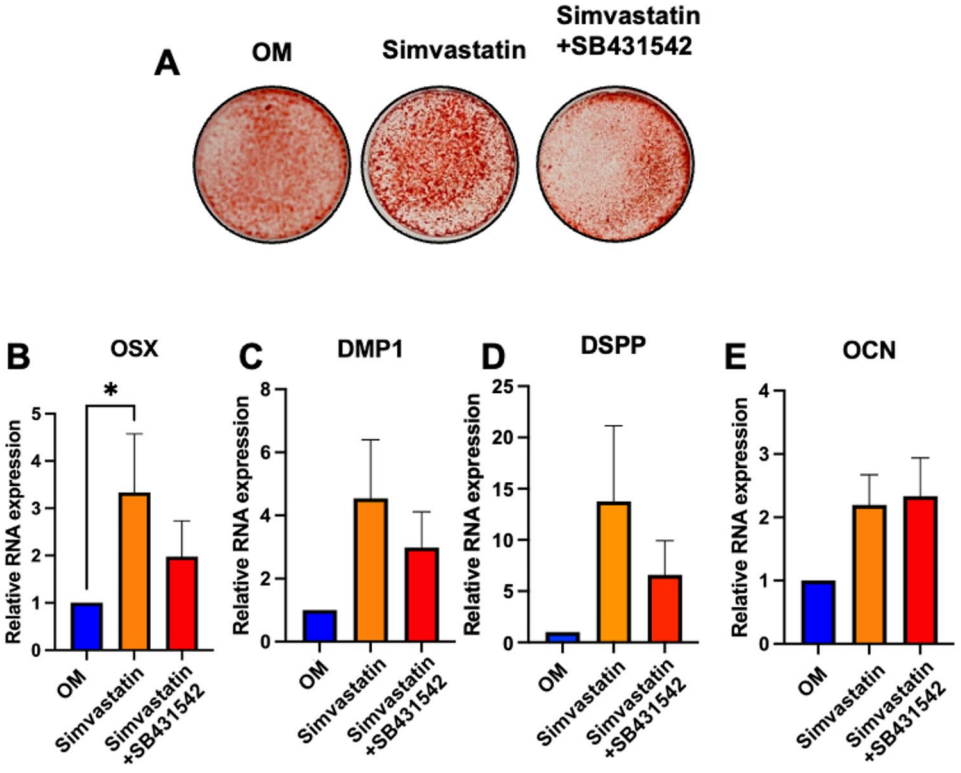

Fig. 4 *TGF-β signalling participated in simvastatin-induced osteogenic differentiation in SCAPs*. Cells were pretreated with SB431542 30 min prior to simvastatin treatment. (**A**) Alizarin Red S staining was utilised to detect mineral deposition on days 14 and (**B-E**), The mRNA levels of osteogenic marker genes were determined using a quantitative polymerase chain reaction on day 7. Bars indicate a statistically significant difference. OM; osteogenic induction medium, $*p < 0.05$

simvastatin exhibited more cementum-like tissue on the dentin surface in the subcutaneous implantation model [42]. Simvastatin, when utilised in conjunction with the chitosan-calcium scaffold, demonstrates significant potential in facilitating dentin regeneration. It augmented the formation of mineralised tissues and upregulated the expression of odontoblastic markers, thereby being proposed as a candidate for the regeneration of dental pulp [43]. The prolonged release of simvastatin from the scaffold enhances its chemoattractant capabilities, thereby amplifying its efficacy in dentin regeneration [43]. Further, calcium phosphate/calcium sulfate biphasic incorporated with simvastatin-induced dentin bridge formation similar to MTA treatment in a pulp capping setting in a dog model [44]. Incorporated simvastatin with MTA led to slightly higher dentin formation with continuous dentin bridge formation compared to MTA alone. However, a higher inflammation score was noted in the simvastatin-incorporated group at an early time point, even though there was no statistically significant difference [45]. In addition to this evidence, the present study added information regarding the osteogenic inductive effects of simvastatin on SCAPs.

This finding highlights the potential of simvastatin in promoting dentin regeneration and dental pulp tissue healing, suggesting its role as an adjunct in endodontic therapies, such as cell-based regenerative endodontics. This technique involves the direct introduction of stem cells into the pulp-root canal space and shows promising results in the regeneration of the dentin-pulp complex in umbilical cord MSCs and DPSCs [46, 47]. SCAPs exposed to simvastatin may be well-suited for this approach. As this technique reduces the reliance on stem cell proliferation and migration, simvastatin's ability to limit SCAP proliferation and migration while enhancing osteogenic differentiation makes it particularly suitable for this application. Moreover, Further investigations should focus on testing simvastatin incorporated with a scaffold to facilitate controlled release, ensuring both optimal concentration maintenance and targeted delivery, or conducting in vivo studies to validate its regenerative effects in clinically relevant environments.

However, experiments utilizing gene knockdown of TGF-β or MAPK targets will be required to further confirm the involvement of these pathways. Furthermore, further in vivo studies are necessary before clinical applications to potentially prove the concept of using simvastatin in regenerative endodontics, focusing on interaction with other regenerative materials to fully understand its potential benefits and optimise therapeutic approaches.

Conclusion

The present study elucidates the effects of simvastatin on SCAPs, particularly emphasising its role in osteogenic differentiation. Findings indicate that simvastatin treatment leads to upregulating genes associated with key osteogenic pathways, such as the TGF-β, FoxO, and MAPK signalling pathways, while concurrently downregulating genes linked to cell proliferation and apoptosis. The enhanced osteogenic differentiation observed in simvastatin-treated SCAPs occurred in a dose-dependent manner. This regulatory mechanism may have manifested through the TGF-β signalling. However, the findings also underscore the necessity for further in vivo investigations to elucidate the therapeutic implications of simvastatin in regenerative endodontics.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-025-05721-z.

Supplementary Material 1

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Author contributions
PR contributed to conceptual design, data acquisition, interpretation, and visualisation. SP, PJ, and CK contributed to data acquisition and data interpretation. UC and LS contributed to data interpretation. TO contributed to conceptual design, data interpretation, project management and manuscript drafting. All authors contributed to the critically revised manuscript and provided approval for submission.

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Data availability
All data generated or analysed during this study are included in this published article. The sequencing data were submitted to NCBI's Gene Expression Omnibus (GSE286540).

Declarations

Ethics approval and consent to participate
The protocol is approved by the Human Research Ethical Research Committee, Faculty of Dentistry, Chulalongkorn University (approval No. 062/2022). All methods were performed in accordance with the relevant guidelines and regulations. Informed consent was obtained.

Consent for publication
Not applicable.

Declaration of generative AI and AI-assisted technologies in the writing process
The authors used generative artificial intelligence tools to improve readability and language during the preparation of this work. The authors reviewed and edited the content as needed and took full responsibility for the publication's content.

Competing interests
The authors declare no competing interests.

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