Archives of Oral Biology 182 (2026) 106468

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Inhibition of protein kinase C activity enables mineralization of senescent dental follicle cells with almost no osteogenic differentiation potential

Christian Morsczeck *, Anja Reck, Michela De Pellegrin, Torsten E. Reichert

Department of Oral and Maxillofacial Surgery, University Hospital Regensburg, Franz-Josef-Strauss-Allee 11, Regensburg 93053, Germany

ARTICLE INFO

Keywords:
Dental follicle cells
Senescence
Bone-morphogenetic-protein 2
Collagen 1
Alkaline phosphatase

ABSTRACT

Objective: Dental follicle cell lines with a senescence phenotype have a poor differentiation potential into biomineralizing cells. Previous studies have shown that protein kinase C (PKC) and protein kinase B (AKT) regulate the differentiation of DFCs. This study investigates the extent to which regulation of PKC and AKT can improve the differentiation of dental follicle cells with poor osteogenic potential.

Design: Human senescence dental follicle cells with poor osteogenic differentiation potential were osteogenic differentiated with cell culture media containing dexamethasone or bone morphogenetic protein (BMP) 2 as an inducer. GÖ6976 was used as a PKC inhibitor, and MK-2206 as an AKT inhibitor. The AKT activator SC-79 was also used. Western blot analyses were performed with specific antibodies for the active form of AKT, phosphorylated substrate of PKC and collagen 1. Osteogenic differentiation was quantitatively determined by measuring alkaline phosphatase (ALP) activity and biomineralization using alizarin staining. The gene expression of sclerostin (SOST) and PTHLH was quantitatively determined using real-time RT-PCRs.

Results: The results showed that both the inhibitor MK-2206 inhibits AKT and the activator SC-79 can activate AKT in DFCs. Only inhibition of AKT slightly but significantly enhanced osteogenic differentiation. While inhibition of PKC activity apparently only occurred from day 14 of differentiation using the inhibitor GÖ6976, PKC inhibition promoted osteogenic differentiation and inhibits the expression of SOST and Parathyroid hormone-related protein (PTHLH).

Conclusion: Our results suggest that the addition of GÖ6976 is an efficient method to induce biomineralization in senescent DFCs.

1. Introduction

Cells can be obtained from the dental follicle of the tooth germ, which is involved not only in tooth eruption but also in the development of the tooth attachment apparatus, which contains biomineralizing cells and fibroblasts that form connective tissue, also known as periodontal ligament (Cahill & Marks, 1980; Marks et al., 1983; Morsczeck et al., 2005; Yi et al., 2022). Dental follicle cells (DFCs) are therefore not exclusively precursor cells for alveolar osteoblasts, which are highly desirable for cell therapies in bone augmentation (Kemoun et al., 2007; Morsczeck et al., 2005). In addition, osteogenic differentiation in particular is suppressed when DFCs age and undergo cellular senescence (Morsczeck et al., 2016). The differentiation of DFCs into fibroblasts, which form connective tissue and cannot mineralize, is therefore highly prone to and poses a major problem for bone tissue engineering. It would therefore be desirable to specifically support osteogenic differentiation.

Protein kinases of signaling pathways such as the protein kinase B (AKT) is involved in the molecular process of osteogenic differentiation (Pieles et al., 2021). For example, previous work showed that AKT is induced during osteogenic differentiation and maintains alkaline phosphatase activity (Viale-Bouroncle et al., 2015). Interestingly, there seems to be a general relationship between AKT and the Bone Morphogenetic Protein (BMP) signaling pathway in DFCs and other cell types (Luo et al., 2015; Pieles et al., 2021; Viale-Bouroncle et al., 2015). However, the AKT activity is downregulated in later stages of differentiation during mineralization. Therefore, inhibition of AKT with MK-2206 induced mineralization of DFCs (Pieles et al., 2021). The same study also showed that AKT was activated after inhibition of protein kinase C (PKC). However, it appears to be more important that inhibition of PKC leads to significantly enhanced mineralization, while activation of PKC leads to inhibition after long-term cultivation in osteogenic differentiation medium (Pieles et al., 2021). Hence, both PKC

* Corresponding author.
E-mail address: christian.morsczeck@ukr.de (C. Morsczeck).

https://doi.org/10.1016/j.archoralbio.2025.106468
Received 28 July 2025; Received in revised form 28 November 2025; Accepted 30 November 2025
Available online 2 December 2025
0003-9969/© 2025 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

and AKT could be interesting targets for improving the differentiation of DFCs into biomineralizing cells. Therefore, this study investigated whether and to what extent activation or inhibition of AKT and inhibition of PKC improves the differentiation of DFCs with a very low osteogenic differentiation potential. Previous studies have shown that induction of cellular senescence in dental follicle cells inhibits osteogenic differentiation (Morsczeck et al., 2016). In this study, we investigated a cell line that undergoes senescence at a very early stage and exhibits virtually no osteogenic differentiation potential. We were able to demonstrate that differentiation could be induced by inhibiting PKC. We investigated alkaline phosphatase activity and mineralization of DFCs as well as the expression of the genes for sclerostin and PTHLH, which are negatively correlated with the expression of osteogenic differentiation markers of DFCs (De Pellegrin et al., 2024; Klingelhöffer et al., 2016).

2. Materials and methods

2.1. Cell culture and osteogenic differentiation

Human dental follicle cells (DFCs) were acquired from Cytion and cultivated in cell culture medium (abbreviated: DMEM) consisting of Dulbecco's modified Eagle's medium F12 with L-glutamin (DMEM-F12; Sigma-Aldrich) supplemented with 5 % fetal bovine serum (FBS; Sigma-Aldrich), HEPES (15 mM) and 1 % penicillin/streptomycin. Due to the commercial nature of the acquisition, we were unable to determine the exact population doubling level. The cell passage was specified for this purpose. Cells at various cell passages were used for the experiments. AKT inhibitor MK-2206 (200 nM), PKC inhibitor GÖ6976 (100 nM) and AKT activator SC-79 (10 μM) were purchased from Merck (Pieles et al., 2021). A control cell line was purchased from All cells and cultured in standard medium (abbreviated: SM) consisting of DMEM with L-glutamin (Sigma-Aldrich) supplemented with 10 % fetal bovine serum (FBS; Sigma-Aldrich), HEPES (15 mM) and 1 % penicillin/streptomycin.

For osteogenic differentiation DFCs were cultured to subconfluence (ca. 70 %-80 %) in standard cell culture medium (see above) before stimulating with osteogenic differentiation medium that consists of DMEM-based cell culture medium with 2 % fetal bovine serum (Sigma-Aldrich), 100 μM ascorbic acid 2-phosphate, 10 mM KH_2PO_4, HEPES (20 mM). To induce osteogenic differentiation, either 10^{-8} M dexamethasone (abbreviated: ODM) or alternatively 50 ng/ml bone morphogenetic protein (BMP2; Biomol) was used (abbreviated: BMP2). Cell culture media were changed every 3–4 days to ensure the effectiveness of inducers that are not stable in cell culture. Differentiation of DFCs was determined by gene expression of osteogenic markers, alkaline phosphatase activity and alizarin red staining.

2.2. Alkaline phosphatase activity (ALP)

The activity of ALP after 7 days of osteogenic differentiation was measured following brief description. The cells were lysed (Triton-X solution) and the lysates were assayed for ALP activity with a solution containing p-nitrophenyl phosphate (60 min at 37°C). The production of p-nitrophenol was measured spectrophotometrically at 415 nm. ALP activity values were calibrated to a sample from the osteogenic differentiation group without simvastatin (ODM). The measured values of the ALP test were related to the measured values (490 nm) for the protein concentration determined with the BCA test (Thermo-Fisher). The ALP activity is expressed as the quotient of the optical densities (415 nm/490 nm). The same volumes of lysate were always used to determine the values of the different samples.

2.3. Alizarin red staining

Differentiation of DFCs was stopped after 4 weeks by fixation, and mineralization of the extracellular matrix was detected using alizarin red staining. Fixation was performed with 4 % formalin, and the mineralized areas were subsequently stained with alizarin red solution (Merck). Quantification of alizarin staining was achieved by dissolving alizarin red crystals in cetylpyridinium chloride and measuring the optical density (595 nm).

2.4. Real-time RT-PCR

Total RNA from DFCs was isolated using the RNeasy isolation kit (Qiagen). The quality of the total RNA was checked using spectrophotometric ratios (A260/A280≥2). cDNA synthesis was performed using 500 ng total RNA and the iScript™ Advanced cDNA Synthesis Kit for qRT-PCR (Biorad) according to the manufacturer's protocol. For a PCR test, 1/20 of the synthesized cDNA was used as a template. PCRs were performed using SsoAdvanced Universal Probes Supermix (Biorad) on the StepOne real-time PCR instrument (Life Technology). Primers for osteogenic differentiation markers were purchased from Bio-Rad. Gene expression of the RPS18 gene (housekeeper gene) was used for normalization. For relative gene expression (calibration), a control group of DFCs cultured in standard medium was used (relative gene expression = 1). Calculations were performed using the previously described methods (Winer et al., 1999).

2.5. Determination of senescent DFCs using a β-galactosidase activity assay

As a senescence assay, β-galactosidase activity was visualized in the cells. The Senescence β-Galactosidase Staining Kit (Cell Signaling Technology, Beverly, MA, USA) was used as previously described (Morsczeck et al., 2016). The cells were made visible under a light microscope and positively stained cells were counted. The count was blinded. At least 400 cells from at least 4 fields of vision, randomly selected on the culture dish, were counted.

2.6. Western-blot analyses

Protein samples were isolated from DFCs and 8 μg of each protein sample were separated by polyacrylamide gel electrophoresis and blotted onto a nitrocellulose membrane as previously described (Pieles et al., 2021; Viale-Bouroncle et al., 2015). The primary antibody for collagen 1 was obtained from Thermo-Fisher Scientific. Primary antibodies for phosphorylated AKT (pAKT) and phosphorylated PKC substrate were purchased from Cell Signaling. Secondary antibodies were detected by chemiluminescence using the ChemiDoc Imaging System (BioRad). For control total protein content was estimated using the stain-free method (Biorad).

2.7. Cell population doubling time

To determine the cell doubling time, the cell count was recorded at two time points, 2 days apart, in the cell culture using the Cell Counting Kit (Dojindo). The cell doubling time was calculated using the following formula: $PDT = t * \ln(2)/\ln(N_t/N_0)$; t: duration of cell culture; N_0: cell number at the beginning of the cell culture; N_t: cell number at the end of the cell culture.

2.8. Statistics

The one-way ANOVA with Tukey's post-hoc tests or alternatively the unpaired Student's tTest were applied. A p-value below 0.05 was considered as significant (*).

3. Results

The selected DFC cell line showed no mineralization after six passages following osteogenic differentiation (Fig. 1 A). In contrast, another

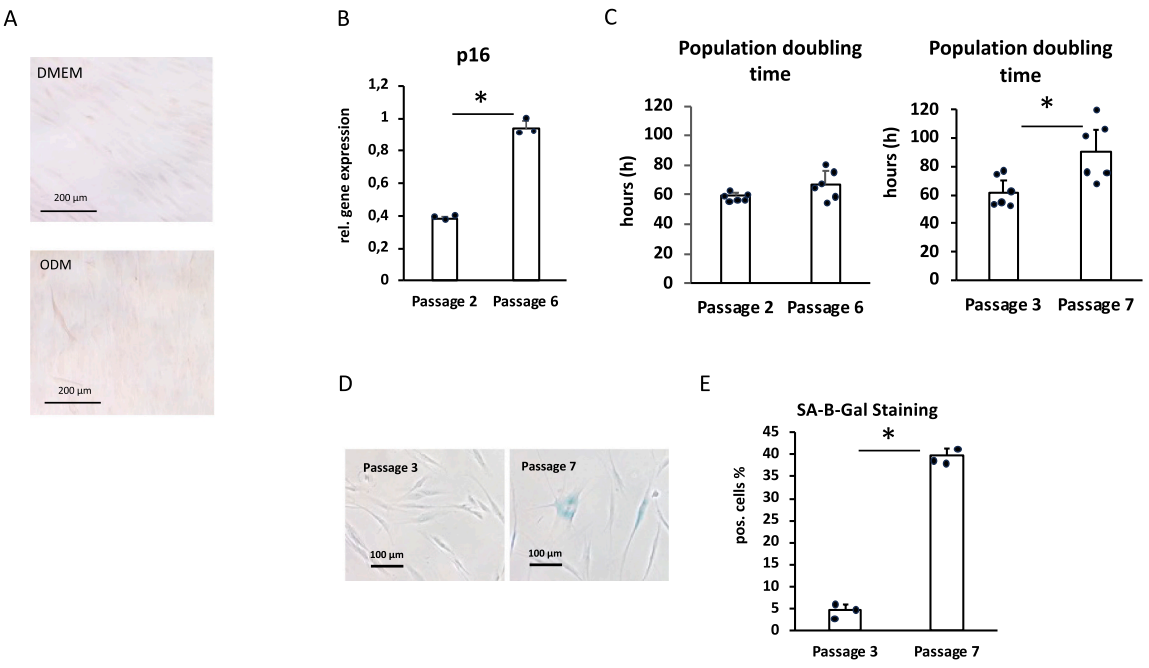

Fig. 1. (A) Alizarin red staining showed that DFCs in passage 6 were poorly mineralized after 4 weeks of treatment in osteogenic differentiation medium (ODM). DFCs in standard medium (DMEM) served as controls. (B) Gene expression of the senescence marker in DFCs in passage 2 and passage 6. The columns present the mean ± SD (n = 3). An asterisk indicates a significant difference ($p < 0.05$). (C) Population doubling time determination in DFCs in passages 2, 3, 6, and 7. The columns present the mean ± SD (n = 6). An asterisk indicates a significant difference ($p < 0.05$). (D, E) β-galactosidase assay with DFCs after culture in passages 3 and 7. Cells at passage 7 show typical senescence features. The columns present the mean ± SD (n = 3). An asterisk indicates a significant difference ($p < 0.05$).

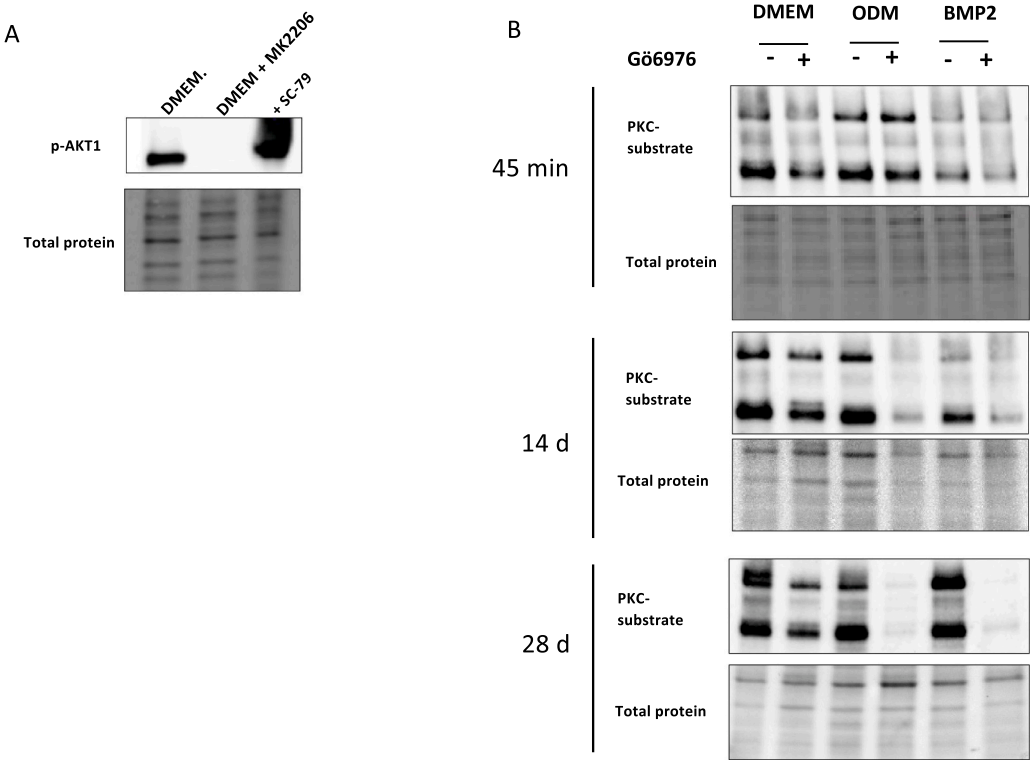

Fig. 2. (A) The efficacy of the AKT inhibitor MK-2206 and the AKT activator SC-79 was demonstrated in a Western blot analysis using a specific antibody for the activated form of the enzyme AKT1 (p-AKT1). The cells were cultured for 45 min; untreated DFCs served as controls. Total protein staining was used for loading control. (B) The efficacy of the PKC inhibitor GÖ6976 was demonstrated by Western blot analysis using a specific antibody against the phosphorylated substrates of PKC (PKC Substrate). Cells were cultured for 45 min, 14 days, and 28 days in standard medium or in osteogenic differentiation media containing dexemethasone (ODM) or BMP2 (BMP2), either with or without GÖ6976. Total protein staining served as a loading control.

cell line was able to mineralize at passage 6 (Figure S1A). These cells exhibited a senescent phenotype with increased expression of the senescence marker P16 (Fig. 1B). The measured cell doubling time, which was significantly higher than in comparable cell lines at passage 6 (Figure S1B), was also increased compared to cells in passage 2 or 3 (Fig. 1 C). A significantly increased doubling time was also observed in passage 7 (Fig. 1 C). In passage 7, cell morphology was markedly enlarged, and the expression of the senescence marker β-galactosidase was significantly increased (Fig. 1D, E). In comparison, a similar senescence phenotype was not detectable in another cell line used as a control at passage 7, but only at a significantly higher passage (Figure S1C). Thus, these DFCs show signs of senescence with limited differentiation potential.

Next, we investigated the extent to which AKT is regulated after treatment with the AKT inhibitor MK-2206 and the AKT activator SC-79. For this purpose, a western blot analysis was performed against the activated form of AKT (pAKT) after 45 min of treatment (Fig. 2 A). Here, the band for the activated form of AKT disappeared after treatment with the inhibitor MK-2206 but a strong induction was observed with the AKT activator SC-79 after 45 min. In order to characterize the inhibitor for PKC, it was investigated to what extent the phosphorylation of the substrate of PKC was inhibited (Fig. 2B). The effects of the PKC inhibitor on the banding pattern were visible after 45 min in standard medium, but not in osteogenic differentiation medium. However, the inhibitor GÖ6976 led to a very significant decrease in phosphorylated proteins 14 and 28 days after induction of osteogenic differentiation (Fig. 2B). At these time points, the effects of GÖ6976 were significantly less visible in standard medium.

To investigate the influence of AKT on differentiation, DFCs were treated with MK-2206 and SC-79 (Fig. 3). After 7 days of osteogenic differentiation, ALP activity was estimated (Fig. 3A) and after 28 days, mineralization was determined (Fig. 3B). ALP activity was significantly upregulated after induction of osteogenic differentiation, but was lower after AKT inhibition and remained unchanged after AKT induction (Fig. 3A). The agreement of the results obtained with osteogenic differentiation medium with dexamethasone or with BMP-2 is worth mentioning. In the next step, we investigated the extent to which the AKT inhibitor and the AKT inducer influence the mineralization of DFCs (Fig. 3B). Interestingly, only the AKT inhibitor induced mineralization slightly but significantly in the dexamethasone-based differentiation medium. However, mineralization was not visually visible after alizarin staining and the measured values are very low. However, the AKT activator SC-79 had no effect on the alizarin red staining.

In the next experiment, the PKC inhibitor GÖ6976 was used (Fig. 4). Inhibition of PKC led to increased ALP activity after induction of differentiation with ODM containing dexamethasone (Fig. 4A). The PKC inhibitor was also able to induce mineralization of DFCs after long-term cultures with ODM and BMP2, which was not possible with standard differentiation media (Fig. 4B). In addition, the protein expression of the osteogenic differentiation marker collagen type I increased after treatment with GÖ6976 (Fig. 5 A). Previous studies have indicated that parathyroid hormone like hormone (PTHLH) and sclerostin (SOST) inhibit osteogenic differentiation of DFCs (De Pellegrin et al., 2024; Klingelhöffer et al., 2016). Interestingly, it has been shown that GÖ6976 exerts its mineralization-promoting effect, among other things, by sclerostin inhibition (De Pellegrin et al., 2024). Our results showed that PKC inhibition inhibits the gene expression of P16, PTHLH and sclerostin (Fig. 5B). These results demonstrate that the PKC inhibitor GÖ6976 improves mineralization in senescent DFCs, probably through inhibition of SOST, PTHLH and P16.

4. Discussion

The induction of cellular senescence and its significance for osteogenic differentiation has been demonstrated previously (Morsczeck, 2021; Morsczeck et al., 2016). However, in the cell line used in this study, senescence occurred after only 6 passages, rather than 12 passages or later as in other DFCs (Morsczeck et al., 2016). Since the induction of senescence is a problem in the proliferation of adult stem cells and this poses a problem for cell therapies, ways must be sought to suppress the effects of senescence on osteogenic differentiation. Therefore, our question was to what extent we might be able to reactivate differentiation through the regulation of AKT and PKC. This present study also extends our previous studies that investigated the role of PKC

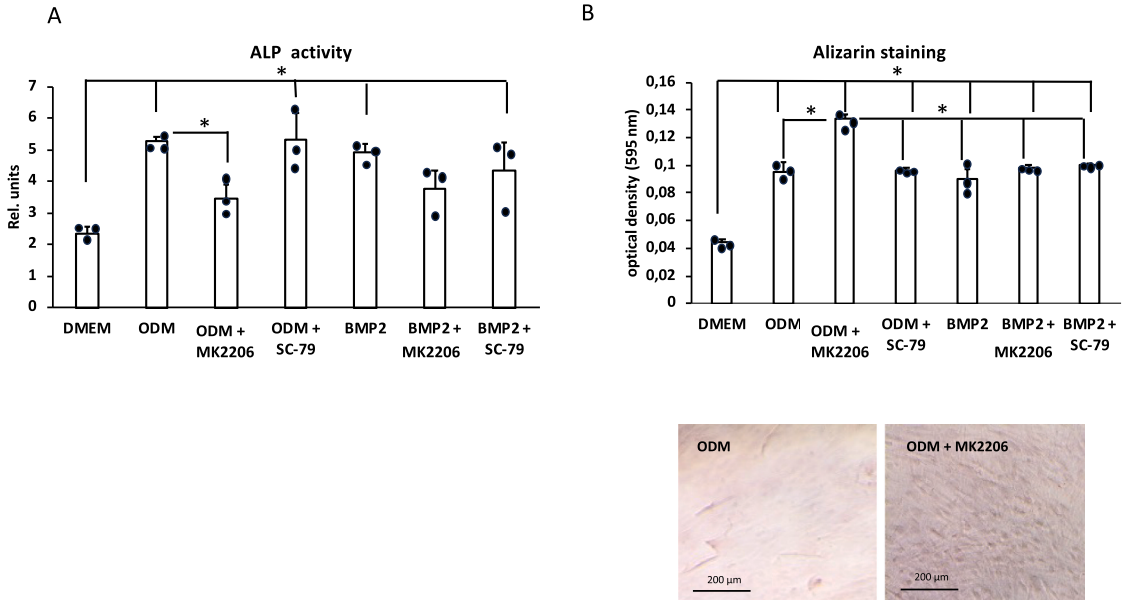

Fig. 3. Analysis of ALP activity and alizarin red staining was used to determine the extent to which treatment with MK-2206 or SC-79 promoted or inhibited osteogenic differentiation of DFCs, which was evaluated after 7 days using ALP activity (A) and after 28 days using alizarin staining (B). Cells were cultured with osteogenic differentiation media containing dexamethasone (ODM) or BMP2 (BMP2) as inducers. For control cells were cultivated in standard medium (DMEM). Columns represent average + /- standard deviation (n = 3). Asterisks (*) indicate significant differences ($p < 0.05$). The pictures below illustrate the weak mineralization.

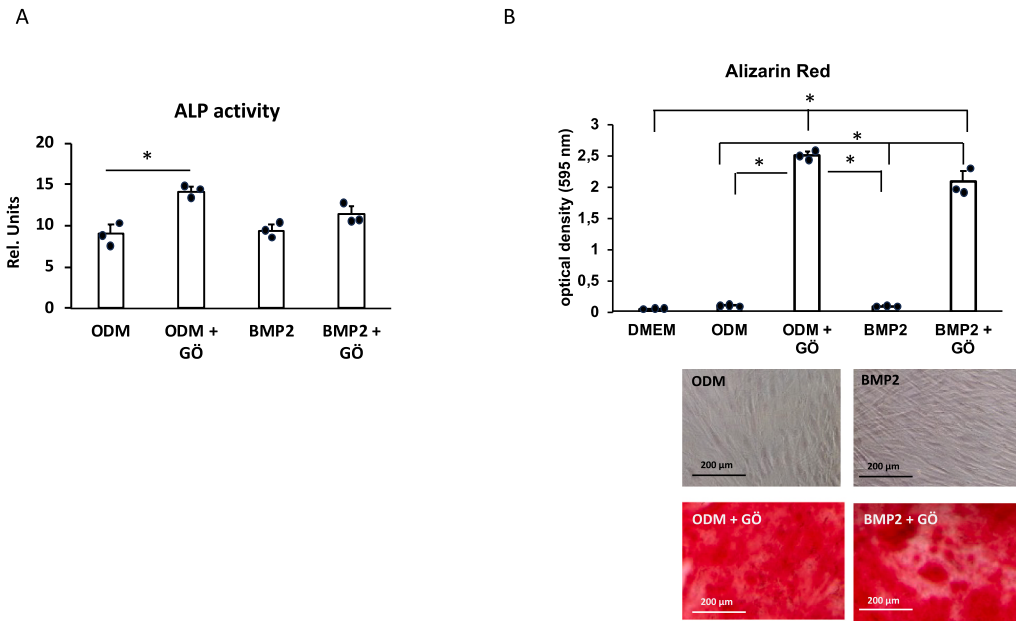

Fig. 4. The extent to which treatment with GÖ6976 (GÖ) promoted or inhibited osteogenic differentiation of DFCs was determined by analyzing ALP activity and alizarin red staining. The evaluation was performed after 7 days based on ALP activity (A) and after 28 days based on alizarin staining (B). Cells were cultured with osteogenic differentiation medium containing dexamethasone (ODM) or BMP2 (BMP2) as inducers. For control cells were cultivated in standard medium (DMEM). The columns represent the mean + /- standard deviation (n = 3). Asterisks (*) indicate significant differences ($p < 0.05$). The images below illustrate the strong induction of mineralization by the PKC inhibitor GÖ6976.

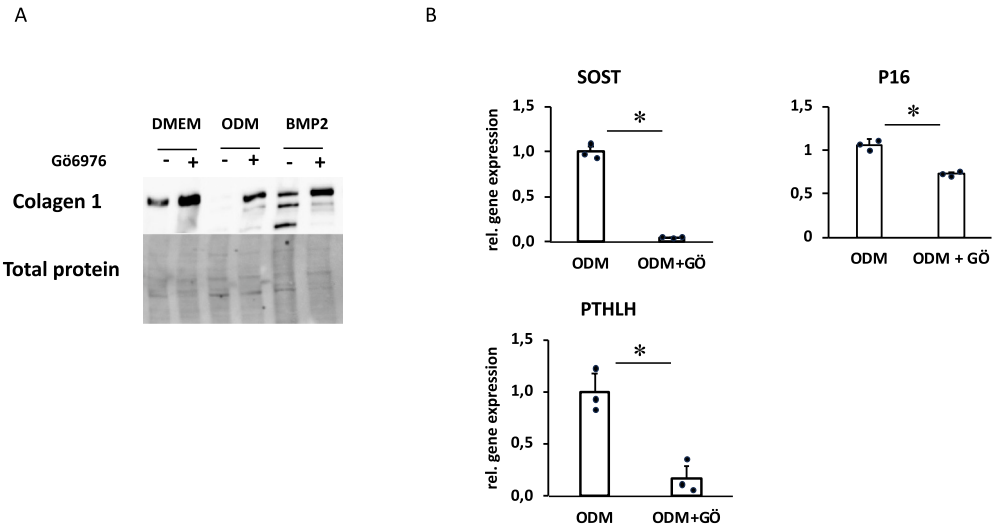

Fig. 5. (A) To demonstrate the induction of osteogenic differentiation by the PKC inhibitor GÖ6976, Western blot analysis was performed with a collagen 1-specific antibody after 14 days of cell culture. The cells were cultured in osteogenic differentiation medium containing dexamethasone (ODM) or BMP2 (BMP2) as inducers. As a control, the cells were cultured in standard medium (DMEM). (B) A real-time analysis of the gene expression of the osteogenic inhibitors PTHLH, P16 and SOST 4 days after induction of osteogenic differentiation in ODM with and without GÖ6976. Columns represent average +/- standard deviation (n = 3). Asterisks (*) indicate significant differences ($p < 0.05$).

and AKT during osteogenic differentiation of DFCs (Pieles et al., 2021; Viale-Bouroncle et al., 2015). We showed that the osteogenic differentiation of DFCs can be improved by the inhibition of PKC and that the regulation of PKC is connected with the activity of AKT and NFκB signaling (Pieles et al., 2021). A connection between PKC and AKT is also known in tumor cells (Hsu et al., 2018) and will be an important starting point for our understanding of osteogenic differentiation of DFCs. This new study suggests that AKT inhibition supports the mineralization of DFCs, as it was shown that AKT inhibition with MK-2206 supported the mineralization of DFCs after induction with BMP2 (Pieles et al., 2021). Interestingly, the AKT-BMP signaling pathway also appears to play a role in differentiation into other functional cells (Luo et al., 2015). However, while we were able to show that AKT inhibition enhances mineralization, studies with various types of adult stem cells have shown a predominantly supportive effect of AKT on osteogenic differentiation (Jia et al., 2024; Li et al., 2022, 2022; Yan et al., 2020). Nevertheless, our results showed that AKT inhibition does not sufficiently promote differentiation, because ALP activity was suppressed, and mineralization was only minimally enhanced. Likewise, AKT activation did not have any effect on differentiation. We must remember that AKT intervenes in very different signaling pathways and biological processes, such as cell proliferation, energy metabolism and DNA repair

(Smith & Eliseev, 2021; Xu et al., 2012). After manipulation of AKT, its downstream signaling pathways are likely controlled by other signaling pathways, as AKT is likely too important for a number of biological processes to be used for our purposes of osteogenic differentiation.

In contrast to the AKT inhibitor, GÖ6976, as an inhibitor of classical PKC such as PKCα, was able to significantly increase the mineralization of senescent DFCs in our study. However, classical PKCs do not inhibit osteogenic differentiation by DFC cell lines, although they do not affect the induction of differentiation (Pieles et al., 2021). One possible mechanism for enhanced osteogenic differentiation could be the regulation of ossification inhibitors. Our results showed that PKC inhibition downregulates the expression of ossification inhibitors. A previous study showed that the osteogenic differentiation inhibitor sclerostin (SOST) is downregulated by GÖ6976 (De Pellegrin et al., 2024). This study showed that SOST was downregulated by GÖ6976 and that the addition of SOST attenuated the stimulatory effect of the PKC inhibitor on differentiation (De Pellegrin et al., 2024). Our recent data suggest that SOST regulation may mediate PKC inhibition-induced mineralization of senescent DFCs. We also showed that GÖ6976 inhibited PTHLH expression, another factor that inhibits osteogenic differentiation of DFCs. (Klingelhöffer et al., 2016). However, the different forms of PTHLH are an important factors for tooth development and appears to be involved in regulating mineralization (Jemtland et al., 2003; Kronenberg et al., 2006; Liu et al., 2020; Ono et al., 2016; Pieles et al., 2020). Although the weak induction of ALP activity by GÖ6976 after differentiation induction with BMP2 is difficult to assess, it suggests that downregulated genes associated with differentiation inhibition, such as SOST and PTHLH, are involved in the reactivation of differentiation in aging DFCs. These genes offer interesting starting points for future investigations. It is also important to note that both osteogenic differentiation and the induction of cellular senescence are associated with the induction of oxidative stress, and that AKT activity is involved in the induction of reactive oxygen species during the osteogenic differentiation of DFCs (Ardura et al., 2017; Estrada et al., 2013; Morsczeck et al., 2023). Interestingly, PKC inhibition also induces AKT activity several days after differentiation induction (Pieles et al., 2021). While the role of AKT activation is complex, it generally supports the activation and nuclear localization of β-catenin during osteogenesis (Pieles et al., 2021; Tang et al., 2020). Furthermore, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which disrupts the mineralization of differentiated DFCs, is also regulated via the PKC/AKT axis (Morsczeck, 2022). Interestingly, a previous study showed that a PKC-dependent signaling pathway supports a cell-free, DFC-derived, matrix vesicle-mediated approach to alveolar bone regeneration (Yi et al., 2022), suggesting a positive role for PKC in the differentiation of alveolar bone progenitor cells. However, we were able to show that PKC inhibition is a good means to enable osteogenic differentiation into cell lines with a low differentiation potential.

Although our study obviously didn't lead to cell rejuvenation, it did show that it's possible to improve mineralization by manipulating the PKC signaling pathway. We think that our work will help to identify other signaling pathways that recover the potential of senescent stem cells. However, the causes of this premature senescence in this cell line remain unclear. Drug-induced senescence in DFCs has been demonstrated in recent years, and the role of oxidative stress has been investigated (Dasi et al., 2023; Meng et al., 2022; Zhai et al., 2017). Future studies may shed light on the changes observed in the cells of our study.

Funding

N/A.

CRediT authorship contribution statement

Reichert Torsten E: Supervision, Resources. **Anja Reck:** Investigation, Data curation. **morsczeck christian:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Michela De Pellegrin:** Investigation, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.archoralbio.2025.106468.

Data Availability

Data will be made available on request.

References

Ardura, J. A., Portal-Nunez, S., Castelbon-Calvo, I., Martinez de Toda, I., De la Fuente, M., & Esbrit, P. (2017). Parathyroid hormone-related protein protects osteoblastic cells from oxidative stress by activation of MKP1 phosphatase. *Journal of Cellular Physiology, 232*(4), 785–796. https://doi.org/10.1002/jcp.25473

Cahill, D. R., & Marks, S. C., Jr. (1980). Tooth eruption: Evidence for the central role of the dental follicle. *Journal of Oral Pathology, 9*(4), 189–200.

Dasi, D., Nallabelli, N., Devalaraju, R., K, N. S., Ghosh, S., Karnati, R., & Sreenivasa Rao, P. (2023). Curcumin attenuates replicative senescence in human dental follicle cells and restores their osteogenic differentiation. *Journal of Oral Biosciences, 65*(4), 371–378. https://doi.org/10.1016/j.job.2023.10.001

De Pellegrin, M., Reck, A., & Morsczeck, C. (2024). Sclerostin inhibits protein kinase C inhibitor GÖ6976 induced osteogenic differentiation of dental follicle cells. *Tissue Cell, 90*, Article 102522. https://doi.org/10.1016/j.tice.2024.102522

Estrada, J. C., Torres, Y., Benguria, A., Dopazo, A., Roche, E., Carrera-Quintanar, L., Perez, R. A., Enriquez, J. A., Torres, R., Ramirez, J. C., Samper, E., & Bernad, A. (2013). Human mesenchymal stem cell-replicative senescence and oxidative stress are closely linked to aneuploidy. *Cell Death Disease, 4*, Article e691. https://doi.org/10.1038/cddis.2013.211

Hsu, A. H., Lum, M. A., Shim, K.-S., Frederick, P. J., Morrison, C. D., Chen, B., Lele, S. M., Sheinin, Y. M., Daikoku, T., Dey, S. K., Leone, G., Black, A. R., & Black, J. D. (2018). Crosstalk between PKCα and PI3K/AKT signaling is tumor suppressive in the endometrium. *Cell Reports, 24*(3), 655–669. https://doi.org/10.1016/j.celrep.2018.06.067

Jemtland, R., Divieti, P., Lee, K., & Segre, G. V. (2003). Hedgehog promotes primary osteoblast differentiation and increases PTHrP mRNA expression and iPTHrP secretion. *Bone, 32*(6), 611–620.

Jia, L., Tian, H., Sun, S., Hao, X., & Wen, Y. (2024). EID3 inhibits the osteogenic differentiation of periodontal ligament stem cells and mediates the signal transduction of TAZ-EID3-AKT/MTOR/ERK. *Biochimica Biophysica Acta Molecular Cell Research, 1871*(3), Article 119662. https://doi.org/10.1016/j.bbamcr.2024.119662

Kemoun, P., Narayanan, A. S., Brunel, G., Salles, J. P., Laurencin-Dalicieux, S., Rue, J., Farges, J. C., Gennero, I., Conte-Auriol, F., Briand-Mesange, F., Gadelorge, M., & Arzate, H. (2007). Human dental follicle cells acquire cementoblast features under stimulation by BMP-2/-7 and enamel matrix derivatives (EMD) in vitro. *Cell Tissue Research, 329*(2), 283–294.

Klingelhöffer, C., Reck, A., Ettl, T., & Morsczeck, C. (2016). The parathyroid hormone-related protein is secreted during the osteogenic differentiation of human dental follicle cells and inhibits the alkaline phosphatase activity and the expression of DLX3. *Tissue and Cell, 48*(4), 334–339. ⟨http://www.sciencedirect.com/science/article/pii/S0040816616300593⟩ (%U).

Kronenberg, H. M. (2006). PTHrP and skeletal development. *Annals of the New York Academy of Sciences, 1068*, 1–13. https://doi.org/10.1196/annals.1346.002

Li, J., Du, H., Ji, X., Chen, Y., Li, Y., Heng, B. C., & Xu, J. (2022). ETV2 promotes osteogenic differentiation of human dental pulp stem cells through the ERK/MAPK and PI3K-Akt signaling pathways. *Stem Cell Research Therapy, 13*(1), 495. https://doi.org/10.1186/s13287-022-03052-2

Liu, C. J., Li, Q. W., Xiao, Q. Y., Gong, P., & Kang, N. (2020). CHD7 Regulates Osteogenic Differentiation of Human Dental Follicle Cells via PTH1R Signaling. *Stem Cells International, 2020*. https://doi.org/Artn%25208882857%252010.1155/2020/8882857.

Luo, W., Zhao, X., Jin, H., Tao, L., Zhu, J., Wang, H., Hemmings, B. A., & Yang, Z. (2015). Akt1 signaling coordinates BMP signaling and β-catenin activity to regulate second heart field progenitor development. *Development, 142*(4), 732–742. https://doi.org/10.1242/dev.119016

Marks, S. C., Jr, Cahill, D. R., & Wise, G. E. (1983). The cytology of the dental follicle and adjacent alveolar bone during tooth eruption in the dog. *The American Journal of Anatomy, 168*(3), 277–289. https://doi.org/10.1002/aja.1001680303

Meng, Z. S., Liu, J. C., Feng, Z. P., Guo, S. L., Wang, M. Z., Wang, Z., Li, Z., Li, H. J., & Sui, L. (2022). -Acetylcysteine regulates dental follicle stem cell osteogenesis and

alveolar bone repair via ROS scavenging. *Stem Cell Research Therapy, 13*(1). https://doi.org/ARTN%2520466%252010.1186/s13287-022-03161-y.

Morsczeck, C. (2021). Effects of cellular senescence on dental follicle cells. *Pharmacology, 106*(3–4), 137–142. https://doi.org/10.1159/000510014

Morsczeck, C. (2022). Mechanisms during osteogenic differentiation in human dental follicle cells. *International Journal of Molecular Sciences, 23*(11). https://doi.org/ARTN%25205945%252010.3390/ijms23115945.

Morsczeck, C., Gotz, W., Schierholz, J., Zeilhofer, F., Kuhn, U., Mohl, C., Sippel, C., & Hoffmann, K. H. (2005). Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biology, 24*(2), 155–165.

Morsczeck, C., Gresser, J., & Ettl, T. (2016). The induction of cellular senescence in dental follicle cells inhibits the osteogenic differentiation. *Molecular and Cellular Biochemistry, 417*(1–2), 334–339. https://doi.org/10.1007/s11010-016-2708-z

Morsczeck, C., Pieles, O., & Beck, H.-C. (2023). Analysis of the phosphoproteome in human dental follicle cells during osteogenic differentiation. *European Journal of Oral Sciences, 131*(5–6), Article e12952. https://doi.org/10.1111/eos.12952

Ono, W., Sakagami, N., Nishimori, S., Ono, N., & Kronenberg, H. M. (2016). Parathyroid hormone receptor signalling in osterix-expressing mesenchymal progenitors is essential for tooth root formation. *Nature Communication, 7*, Article 11277. https://doi.org/10.1038/ncomms11277

Pieles, O., Reck, A., & Morsczeck, C. (2020). High endogenous expression of parathyroid hormone-related protein (PTHrP) supports osteogenic differentiation in human dental follicle cells. *Histochemistry and Cell Biology, 154*(4), 397–403. https://doi.org/10.1007/s00418-020-01904-7

Pieles, O., Reichert, T. E., & Morsczeck, C. (2021). Classical isoforms of protein kinase C (PKC) and Akt regulate the osteogenic differentiation of human dental follicle cells via both β-catenin and NF-κB. *Stem Cell Research Therapy, 12*(1), 242. https://doi.org/10.1186/s13287-021-02313-w

Smith, C. O., & Eliseev, R. A. (2021). Energy metabolism during osteogenic differentiation: The role of Akt. *Stem Cells and Development, 30*(3), 149–162. https://doi.org/10.1089/scd.2020.0141

Tang, J., Qing, M. F., Li, M., & Gao, Z. (2020). Dexamethasone inhibits BMP7-induced osteogenic differentiation in rat dental follicle cells via the PI3K/AKT/GSK-3beta/beta-catenin pathway. *International Journal of Medical Sciences, 17*(17), 2663–2672. https://doi.org/10.7150/ijms.44231

Viale-Bouroncle, S., Klingelhöffer, C., Ettl, T., & Morsczeck, C. (2015). The AKT signaling pathway sustains the osteogenic differentiation in human dental follicle cells. *Molecular and Cellular Biochemistry, 406*(1), 199–204. https://doi.org/10.1007/s11010-015-2437-8

Winer, J., Jung, C. K., Shackel, I., & Williams, P. M. (1999). Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. *Analytical Biochemistry, 270*(1), 41–49.

Xu, N., Lao, Y., Zhang, Y., & Gillespie, D. A. (2012). Akt: A double-edged sword in cell proliferation and genome stability. *Journal of Oncology, 2012*, Article 951724. https://doi.org/10.1155/2012/951724

Yan, Y., Zhang, H., Liu, L., Chu, Z., Ge, Y., Wu, J., Liu, Y., & Tang, C. (2020). Periostin reverses high glucose-inhibited osteogenesis of periodontal ligament stem cells via AKT pathway. *Life Sciences, 242*, Article 117184. https://doi.org/10.1016/j.lfs.2019.117184

Yi, G., Zhang, S., Ma, Y., Yang, X., Huo, F., Chen, Y., Yang, B., & Tian, W. (2022). Matrix vesicles from dental follicle cells improve alveolar bone regeneration via activation of the PLC/PKC/MAPK pathway. *Stem Cell Research & Therapy, 13*(1), 41. https://doi.org/10.1186/s13287-022-02721-6

Zhai, Y., Wei, R., Liu, J., Wang, H., Cai, W., Zhao, M., Hu, Y., Wang, S., Yang, T., Liu, X., Yang, J., & Liu, S. (2017). Drug-induced premature senescence model in human dental follicle stem cells. *Oncotarget, 8*(5), 7276–7293. https://doi.org/10.18632/oncotarget.14085