DPY30 Promotes Odontoblast Differentiation

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ABSTRACT

Purpose: DPY30 is a core subunit of SET1/MII complexes, the major histone H3K4 methyltransferases in mammals. It plays an important role in regulating fundamental processes including the determination of cell lineage, growth, and differentiation. Histone-based epigenetic mechanisms are involved in odontoblast differentiation and function. This study aimed to determine the role of DPY30 in the epigenetic regulation of odontoblast differentiation in vitro.

Methods: Primary human dental pulp cells (hDPCs) were used to induce odontoblast differentiation. Odontoblast differentiation and mineralization were determined by gene expression and staining with alkaline phosphate (ALP), and alizarin red (ARS). The effect of DPY30 on odontoblast differentiation and mineralization was determined by the siRNA knockdown of DPY30. The gene expression was analyzed at days 0, 3, 7, 14, and 21 of differentiation by quantitative PCR.

Results: During hDPCs differentiation, the expression of DPY30 increased from day 3 to day 21, along with a progressive increase in the expression of both early (RUNX2, COL1A, DSPP) and late (DMP1, OCN) marker genes. Differentiation of hDPCs to odontoblasts was further confirmed by ALP and ARS staining. Successful knockdown of DPY30 was achieved and confirmed by qPCR. DPY30 knockdown in hDPCs led to a marked reduction in ALP activity and odontoblast differentiation. In addition, the expression of odontogenic marker genes RUNX2, COL1A, DSPP, DMP1, and OCN was significantly reduced by DPY30 depletion.

Conclusion: DPY30 mediates H3K4 methylation and promotes odontoblast differentiation and mineralization. Our results demonstrate the critical role of DPY30 in the epigenetic regulation of odontogenic differentiation of hDPCs.

INTRODUCTION

Dentin is mineralized tissue and the most abundant component of the tooth. Defects in dentin lead to dentin disorders, early tooth exfoliation, or missing teeth. Dentin is produced from odontoblasts, derived from ectomesenchymal cells of human Dental Pulp Cells (hDPCs), which are essential for dentin formation, remodeling, and mineral hemostasis. Histone-based epigenetic mechanisms are involved in odontoblast differentiation and function. Histone H3K4 methylation is one of the most important histone-based epigenetic mechanisms which is generally associated with gene activation. DPY30 as an integral core subunit is essential for the full methylation activity. Previous studies have established a direct role of the DPY30 in facilitating genome-wide H3K4 methylation. Therefore, it is most likely that DPY30 could regulate odontogenesis via regulating H3K4 methylation.

MATERIALS AND METHODS

- We used Primary human dental pulp cells (hDPCs) to induce odontoblast differentiation.
- Odontoblast differentiation and mineralization were determined by gene expression and staining with alkaline phosphate (ALP), and alizarin red (ARS).
- The effect of DPY30 on odontoblast differentiation and mineralization was determined by the siRNA knockdown of DPY30.
- The gene expression was analyzed at days 0, 3, 7, 14, and 21 of differentiation by quantitative PCR.

RESULTS

Assessment of differentiation of hDPCs to odontoblasts done by ALP and ARS staining

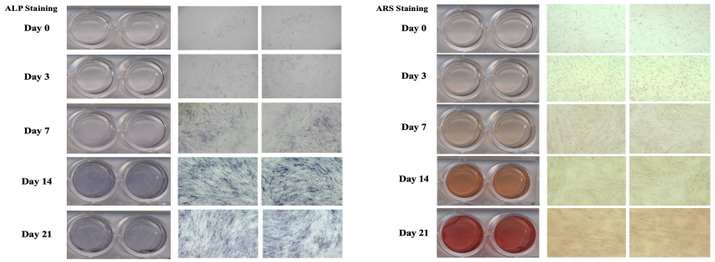

Figure 1: Differentiation and mineralization of odontoblasts. hDPCs were cultured in the osteogenic medium for 0-21 days. A) Odontoblast differentiation was evaluated by ALP. B) ARS staining showing the presence of calcium deposits (in orange-red/red color). Representative images of three different experiments.

During hDPCs differentiation, the expression of DPY30 increased from day 3 to day 21, along with a progressive increase in the expression of both early (RUNX2, COL1A, DSPP) and late (DMP1, OCN) marker genes

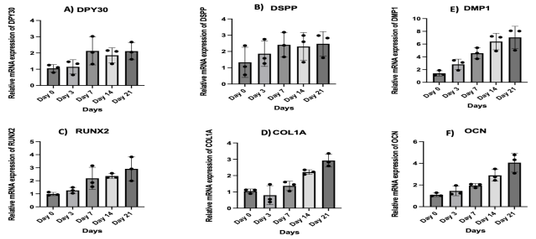

Figure 2: Gene expression of odontoblast-related markers in DPY30 hDPCs. Cells were cultured with the osteogenic medium on days 0, 3, 7, 14, and 21. An increase in gene expression from day 3 was seen in both early and late marker genes. A) Gene expression of DPY30 in hDPCs. B), C), and D) Gene expression of DSPP, RUNX2, and COL1A in hDPCs. E) and F) Gene expression of DMP1 and OCN in hDPCs.

DPY30 knockdown in hDPCs led to a marked reduction in ALP activity and odontoblast differentiation

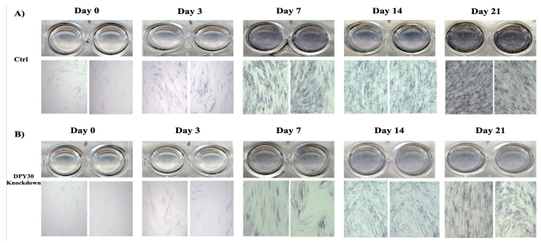

Figure 3: Differentiation of Ctrl and knockout DPY30 hDPCs. Odontoblast differentiation was evaluated in hDPCs cultured in the osteogenic medium for 0-21 days. Odontoblast differentiation was evaluated by ALP. A) Images of ALP staining of odontoblast differentiation of DPY30 Ctrl hDPCs from days 0-21. B) Images of ALP staining of odontoblast differentiation of DPY30 Knockout hDPCs from days 0-21. Representative images of ALP staining of hDPCs of three independent experiments.

RESULTS

DPY30 knockdown in hDPCs led to a reduction in ARS activity.

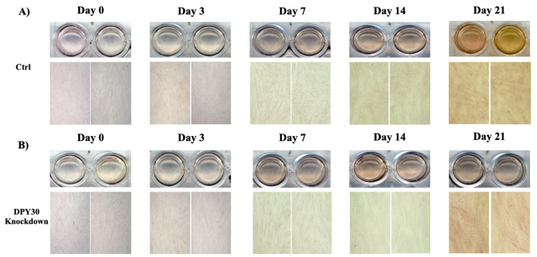

Figure 4: Mineralization of Ctrl and knockout DPY30 hDPCs. Odontoblast mineralization was evaluated in hDPCs cultured in the osteogenic medium for 0-21 days. Odontoblast mineralization was evaluated by ARS. A) Images of ARS staining of odontoblast mineralization of DPY30 Ctrl hDPCs from days 0-21. B) Images of ARS staining of odontoblast mineralization of DPY30 Knockout hDPCs from days 0-21. Representative images of ARS staining of hDPCs of three independent experiments.

Successful knockdown of DPY30 was achieved and confirmed by qPCR.

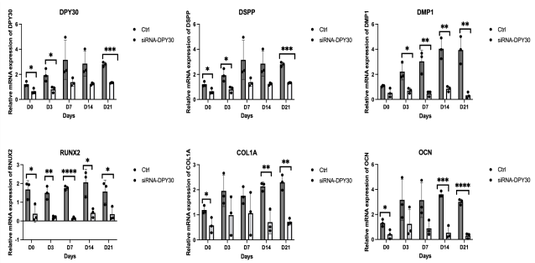

Figure 5: Gene expression of odontoblast-related markers in Ctrl and knockout DPY30 hDPCs. DPY30 regulates the expression of odontoblast-related genes at the transcription level. qPCR analysis of mRNA expression of DPY30, DSPP, RUNX2, COL1A, DMP1, and OCN during odontoblast differentiation from days 0 to 21. Data are mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

CONCLUSION

DPY30 mediates H3K4 methylation and promotes odontoblast differentiation and mineralization. Our results demonstrate the critical role of DPY30 in the epigenetic regulation of odontogenic differentiation of hDPCs. Thus, the proposed study will not only improve our understanding of the epigenetic regulation of dentin formation but also facilitate the design of novel therapeutic approaches for diseases involved in dentin matrix malformation, and skeletal and craniofacial abnormalities. It will also lay a foundation for new and effective therapeutic approaches targeting epigenetic modulators of dentin-related diseases.

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