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The Essential Role of BMP Signaling in Tooth Root Formation

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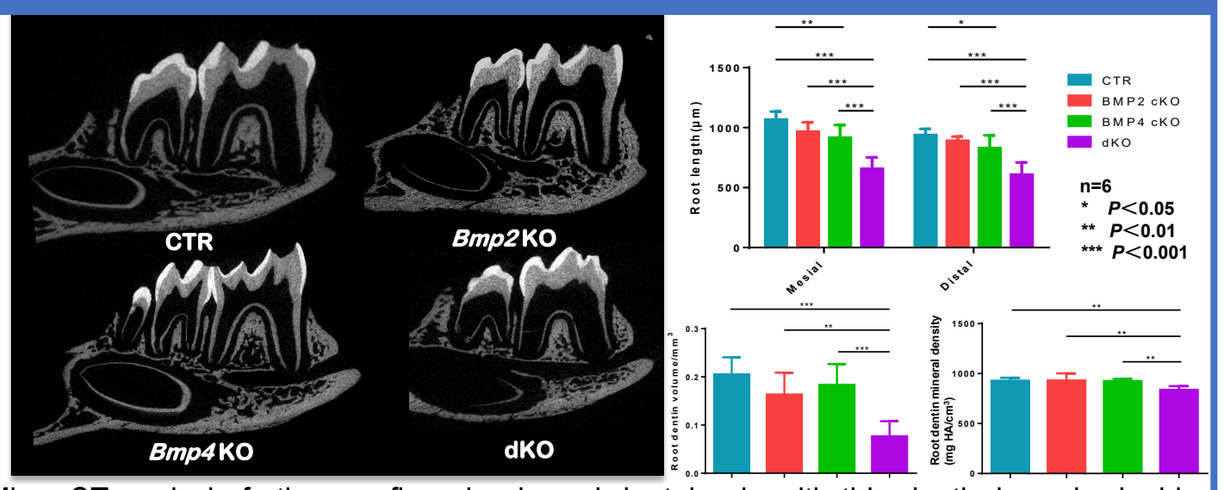
OBJECTIVES

Jawbone and dentin share many common features, although which one evolutionarily comes first is still under debate. The goal of this study was to investigate the role of BMP2 and BMP4 in controlling the fate of pulp cells during molar root formation.

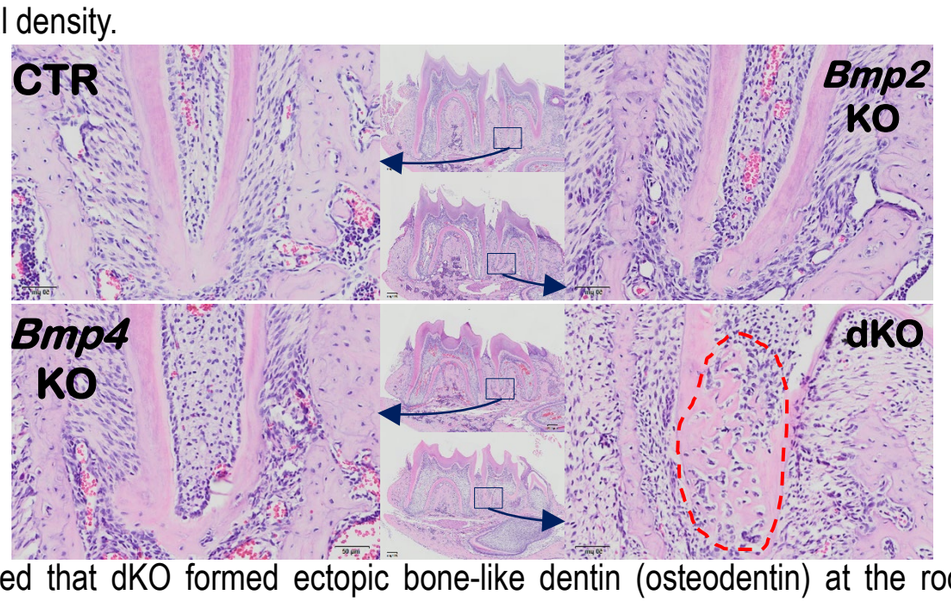
MATERIALS AND METHODS

The *Gli1-CreER* mice were crossed with *Bmp2*$^{flox/flox}$ and *Bmp4*$^{flox/flox}$ mice in *Rosa26-tdTomato* background to specifically inactivate *Bmp2* and/or *Bmp4* (two key BMP ligands) in the dental pulp progenitor cells. A single dose of tamoxifen was injected at postnatal day 5 and animals were harvested at postnatal week 4 with EdU injection 3 hours before sacrifice. The combined approaches of μCT, *in vivo* cell lineage-tracing, histology, SEM, immunostaining, and RNA-seq were used.

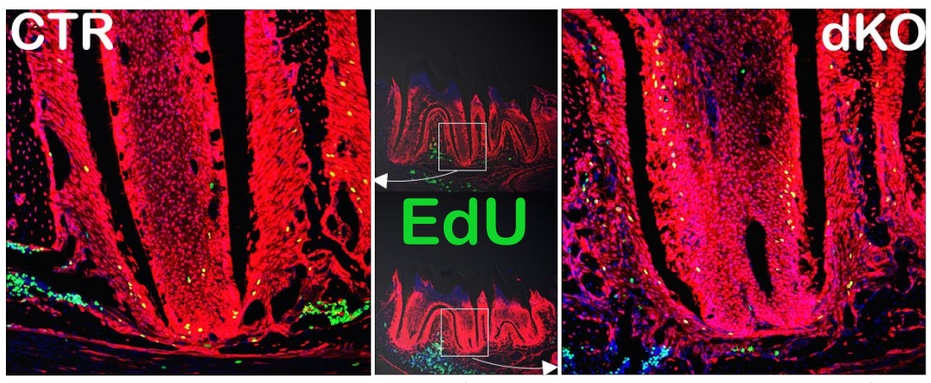
RESULTS



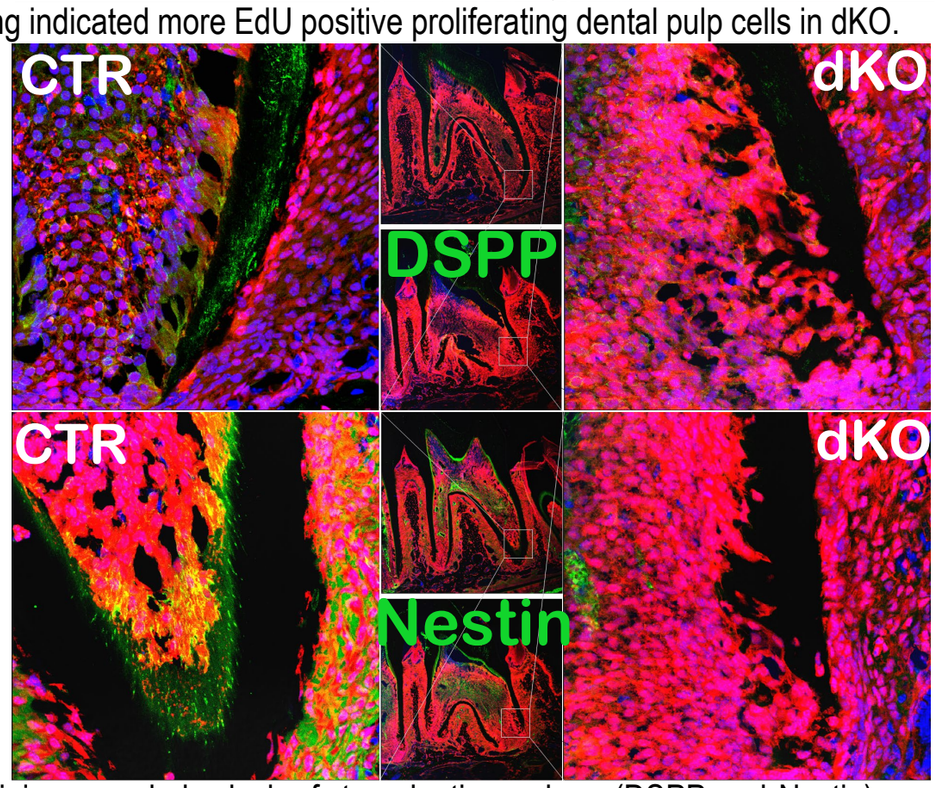
Micro-CT analysis further confirmed enlarged dental pulp with thin dentin layer in double knockout (dKO). Quantitative analyses of μ-CT demonstrated shorter molar roots in dKO when compared to either CTR or single KO, with significantly reduced root dentin volume and mineral density.



H&E showed that dKO formed ectopic bone-like dentin (osteodentin) at the root apical region.

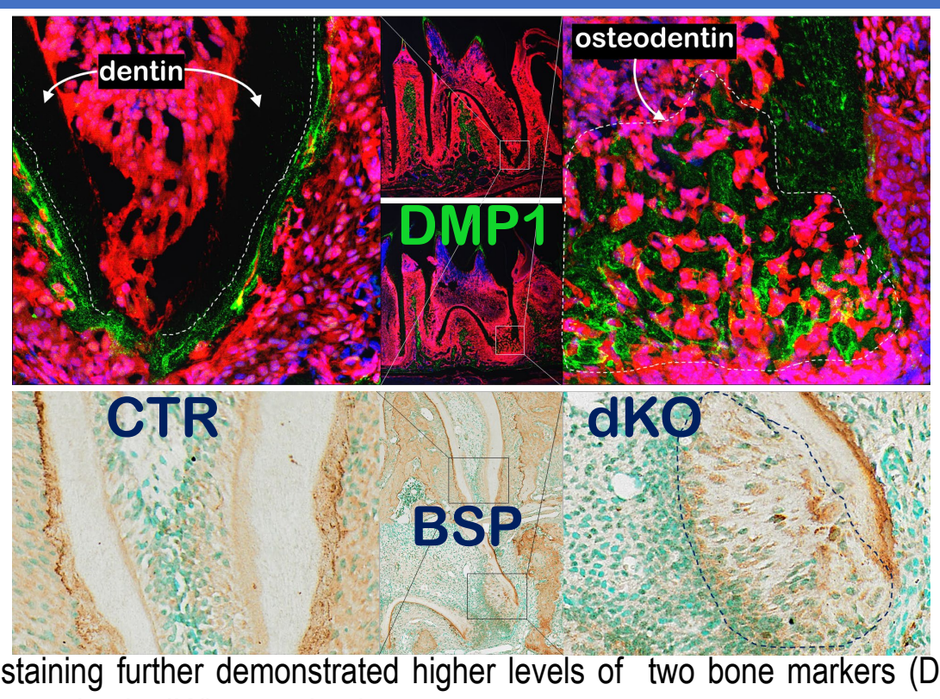


EdU staining indicated more EdU positive proliferating dental pulp cells in dKO.

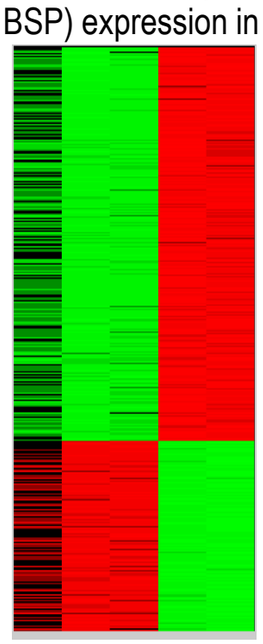


Immunostaining revealed a lack of two dentin markers (DSPP and Nestin) expression in dKO root dentin.

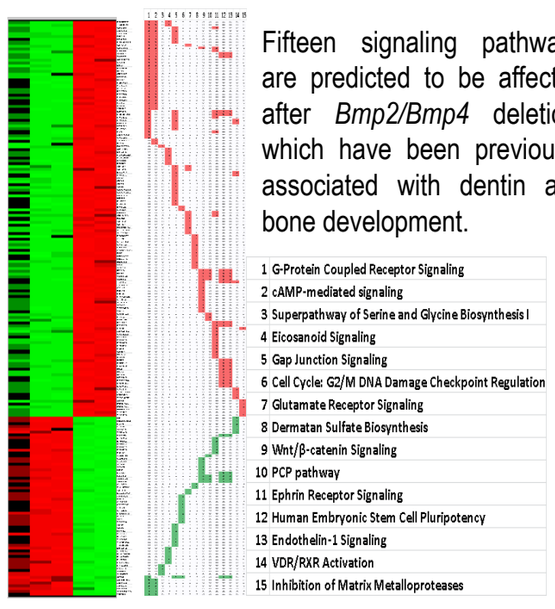
RESULTS



Immunostaining further demonstrated higher levels of two bone markers (DMP1 and BSP) expression in dKO osteodentin.

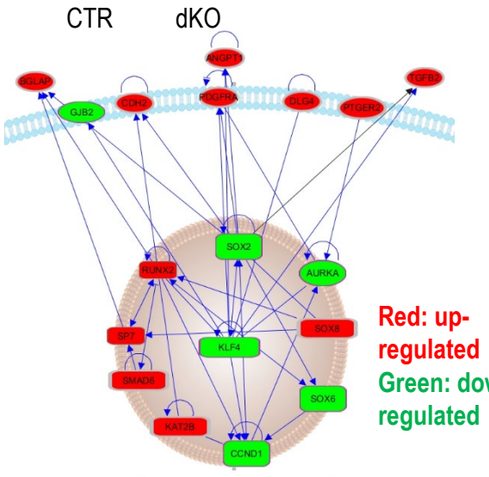


There are 1038 transcripts with differential abundance in dental pulp cells from CTR and dKO, 699 increased and 339 decreased.



Fifteen signaling pathways are predicted to be affected after *Bmp2/Bmp4* deletion, which have been previously associated with dentin and bone development.

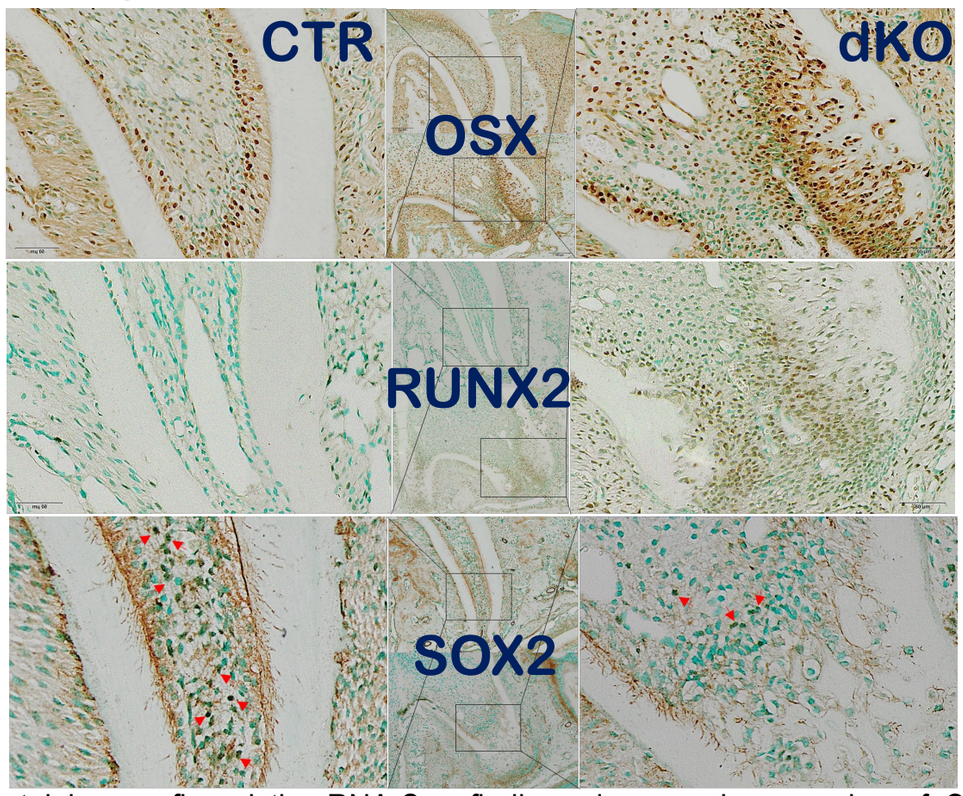
CTR dKO



Red: up-regulated
Green: down-regulated

Since transcription factors play critical roles in cell fate determination, we focused on 10 transcription factors, which are differentially-regulated and associated with dentin and bone formation.

Notably, we found increased expression of osteogenic transcription factors (Runx2 and Sp7 (Osterix)) and decreased expression of odontogenetic transcription factors (Klf4 and Sox2).



Immunostaining confirmed the RNA-Seq findings: increased expression of OSX and RUNX2, but decreased expression of SOX2.

SUMMARY AND CONCLUSION

Our findings demonstrated that BMP signaling (a combined role of BMP2 and BMP4) is essential for determining the cell fate of Gli1^{+} pulp cells during molar root formation, and supported a notion that tooth development occurs after jawbone.

ACKNOWLEDGEMENTS

This study was supported by National Natural Science Foundation of China (82071127 and 81700980) to JW, US NIH grants (R01DE025014 and R01DE025659) to JQF.