Enamel development relies on WDR72 regulation of endocytosis through microtubule organization

Kei Katsura, Judy Zhu, Yukiko Nakano, Yan Zhang, Pamela Den Besten; Department of Oral and Craniofacial Sciences and School of Dentistry, University of California, San Francisco

Introduction

- Amelogenesis Imperfecta (AI) is a genetic disorder that severely affects tooth enamel formation. In both humans and mice, mutations in *Wdr72* cause AI with severely hypocalcified enamel.

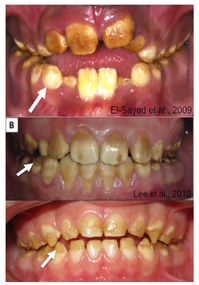


Figure 1. Amelogenesis Imperfecta in patients with *Wdr72* mutations showing hypocalcified enamel phenotypes in all teeth.

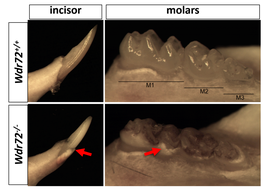


Figure 2. Lower teeth of 6-week-old mice. Wildtype enamel (top) is translucent & hard, while knockout enamel (bottom) is opaque and easily worn away.

- Enamel mineralization requires the removal of amelogenin proteins from the matrix by both being hydrolyzed by secreted proteases and by being taken back up into ameloblasts.
- Adequate amelogenin removal from the matrix is defective in *Wdr72* $^{-/-}$ mice, thus leading to hypocalcified enamel. However, the cell mechanism linking amelogenin removal and mineralization remains unclear.

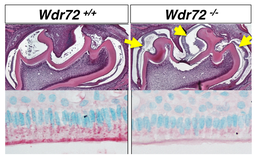


Figure 3. TOP: H&E stains showing retained organic matrix (arrows) in *Wdr72* $^{-/-}$ mice.
BOTTOM: Amelogenin (red) in *Wdr72-/-* ameloblasts is decreased.

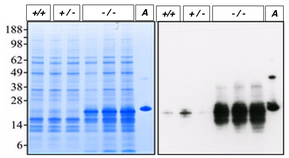


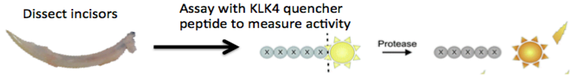
Figure 4. SDS-PAGE (left) & Western Blot (right) show decreased total protein & amelogenin protein, respectively, in *Wdr72-/-* enamel matrices. A, recombinant amelogenin (Wang et al., 2015)

Objective

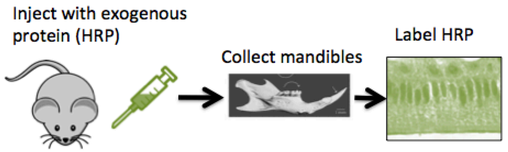
Determine if WDR72 regulates amelogenin removal by directing either (a) secretion of proteases or (b) uptake of extracellular proteins. We further investigated the mechanism by which WDR72 regulates vesicle transport[1].

Methods

KLK4 protease activity was measured in *Wdr72* $^{+/+}$ and *Wdr72* $^{-/-}$ enamel matrices using a quencher peptide. Klk4 transcript levels were verified in *Wdr72* $^{-/-}$ ameloblasts using qPCR.

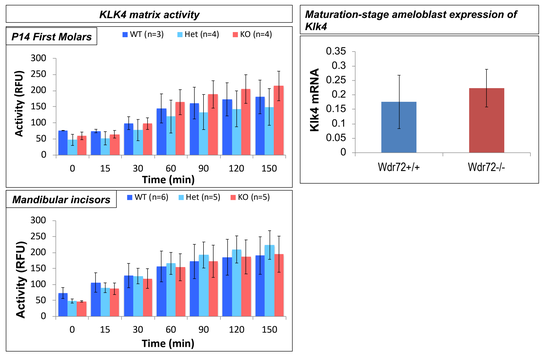


Verification of Wdr72 knockout with CRISPR/Cas9 was performed with DNA Sanger sequencing and WDR72 antibody. Confocal microscopy was used for all cell culture experiments. Transmission electron microscopy was used to assess ameloblast ultrastructure, following fixation and resin embedding. Horseradish peroxidase (HRP) was injected in mice for 15, 30, and 60min to trace constitutive endocytosis.



Results

Figure 5. (left) KLK4 protease activity in WT and KO mice in molars and incisors. **(below)** KLK4 mRNA expression levels normalized with 18S in P14 molars (n=4).



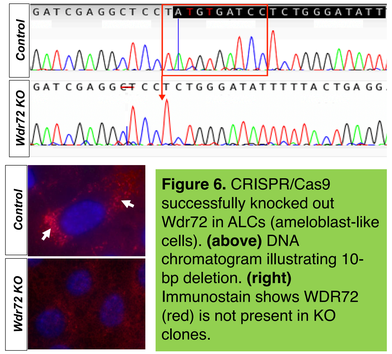
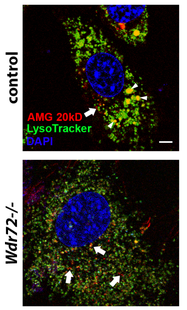


Figure 6. CRISPR/Cas9 successfully knocked out Wdr72 in ALCs (ameloblast-like cells). **(above)** DNA chromatogram illustrating 10-bp deletion. **(right)** Immunostain shows WDR72 (red) is not present in KO clones.

Figure 7. Under time-lapse imaging, control cells internalized amelogenin (red) and co-localized with lysosomes (green). Cells without WDR72 also internalized amelogenin, however, were unable to localize amelogenin to lysosomes.



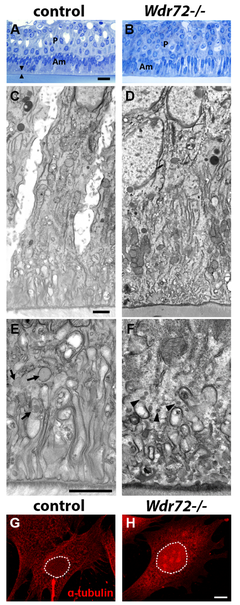
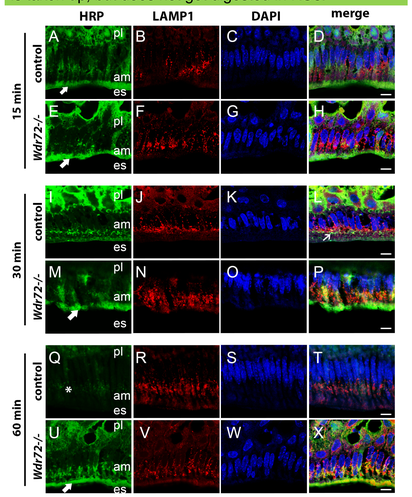


Figure 8. Ultrastructure of KO ameloblasts show disorganized and blunted ruffle-border (D) & smaller vesicles (F). KOs show disrupted tubulin organization (H).

Figure 9. HRP, a marker of constitutive endocytosis, is delayed in uptake and processing in KO ameloblasts. **(top panels)** Controls show HRP (green) uptake and colocalization with LAMP1 (red). **(bottom panels)** HRP is taken up, but does not get digested in KOs.



Conclusions

- WDR72 regulates transport of proteins in the endo-lysosome system.
- This transport may be facilitated by WDR72 function in microtubule organization, indicating this encompasses all disrupted functions in ameloblasts without Wdr72.
- Coupled with our previously published data, we propose a role as a regulator of vesicle membrane formation, possibly in association with Clathrin-coated vesicles.

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UCSF University of California San Francisco School of Dentistry