

Autophagy plays a crucial role in ameloblast differentiation

Chihiro Iwaya and Junichi Iwata, University of Texas Health Science Center at Houston, School of Dentistry, Houston, Texas, USA.

Objectives: Any failure in amelogenesis results in defects in the enamel, a condition known as amelogenesis imperfecta. The epithelial compartment of the developing tooth is called enamel organ and comprises specific cell types, including inner and outer enamel epithelial cells. The inner enamel epithelial cells differentiate into ameloblasts, which go through pre-secretory, secretory, transition, and maturation stages. The objective of this study is to identify the role of autophagy, a major cellular degradation system for proteins and organelles, in ameloblasts.

Methods: Autophagy-deficient and control mice were scanned with a SCANCO vivaCT-40 system at 15- μ m resolution. 3D reconstruction μ CT imaging and consequent analyses were performed using the Dragonfly software [Version 2021.1 for Windows; Object Research Systems Inc., Montreal, Canada]. Histology (immunohistochemistry for autophagy-related molecules, H&E staining, and *In situ* hybridization for genes related to ameloblast differentiation), promoter analysis for a transcription factor binding site and ChIP assays were performed.

Results & Conclusion: At the molecular level, ameloblast differentiation was compromised due to ectopic accumulation and activation of NRF2, a substrate of autophagy. Through bioinformatic analyses, we identified several candidate genes related to amelogenesis imperfecta (*Bcl11b*, *Dlx3*, *Klk4*, *Ltbp3*, *Nectin1*, and *Pax9*). Among them, we experimentally validated that the NRF2 pathway suppressed gene expression of *Bcl11b*, *Dlx3*, *Klk4*, and *Nectin1*. Taken together, our findings indicate that autophagy plays a crucial role in ameloblast differentiation and its defect results in amelogenesis imperfecta through ectopic NRF2 activation.