P6-4

Lin28 contributes to odontogenic differentiation of dental papilla cells during tooth development

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Abstract Odontoblasts are responsible for dentin formation. They originate from dental papilla cells at the late bell stage of tooth development. The cell fate determination of dental papilla cells contributes to giving rise to odontoblasts, which is a key process during dentin formation. Lin28 is a conserved RNA binding protein in eukaryotic cells. Lin28 manifested a strong role in organ development and cell differentiation. However, few studies have probed into its expression during tooth development. By immunohistochemical staining, we have found that Lin28 showed specific expression pattern during mouse first molar development. Lin28 showed continuous high expression in the epithelium from E12.5 to P3. Lin28 was absent in the dental papilla and meanwhile boosted in the odontoblasts from E17.5 and on. Lin28b, the paralog of Lin28, was not detected during tooth development. Lin28 expression level did not change during in vitro odontogenic differentiation of dental papilla cells. But overexpression of Lin28 promoted odontogenic differentiation of dental papilla cells in vitro. The results revealed Lin28's potential role in promoting dental papilla cells' cell fate determination towards odontoblasts.

Introduction

Dentin formation is achieved by odontoblasts, a special type of terminally differentiated cells lying on the outer wall of dental pulps. They originate from dental papilla cells at the late bell stage of tooth development. The cell fate determination of dental papilla cells is responsible for giving rise to odontoblasts, which is a key process during dentin formation, involving a variety of factors and signals. Exploring the key factors and specific mechanisms of this process is a hot spot in the research arena of dentin development. Signaling pathways including TGF-β, Wnt, FGF, IGF, etc., transcriptional factors such as Runx2, Sp7, Klf4, Dlx3, etc. have been unveiled to engage in orchestrating this process.

RNA binding proteins, RBPs, are a diverse class of proteins that are involved in regulating gene expression. Lin28 is a conserved RNA binding protein in eukaryotic cells. It has been unveiled that Lin28 manifested a strong role in organ development and cell differentiation. Lin28 has two paralogs, Lin28 and Lin28b, who share the similar protein domains and functions. Lin28 functions in cytoplasm, while Lin28b in nucleus. However, few studies have probed into its expression during tooth development.

Results

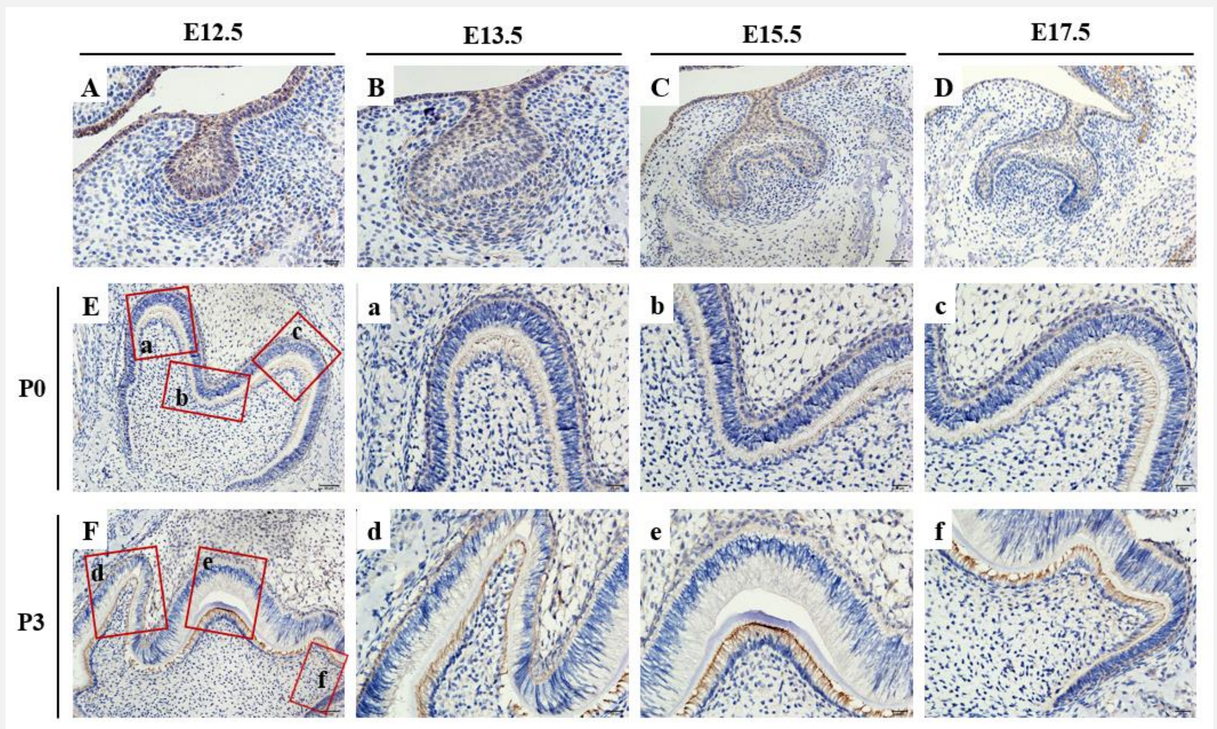

Fig1. A~D.From E12.5 to E17.5, Lin28 was continuously expressed in epithelium, and lowly expressed in mesenchymal. E~F. Lin28 was continuously expression in epithelium. Lin28 was highly expressed in odontoblasts in P0 and P3, while remained low expression level in dental papilla.

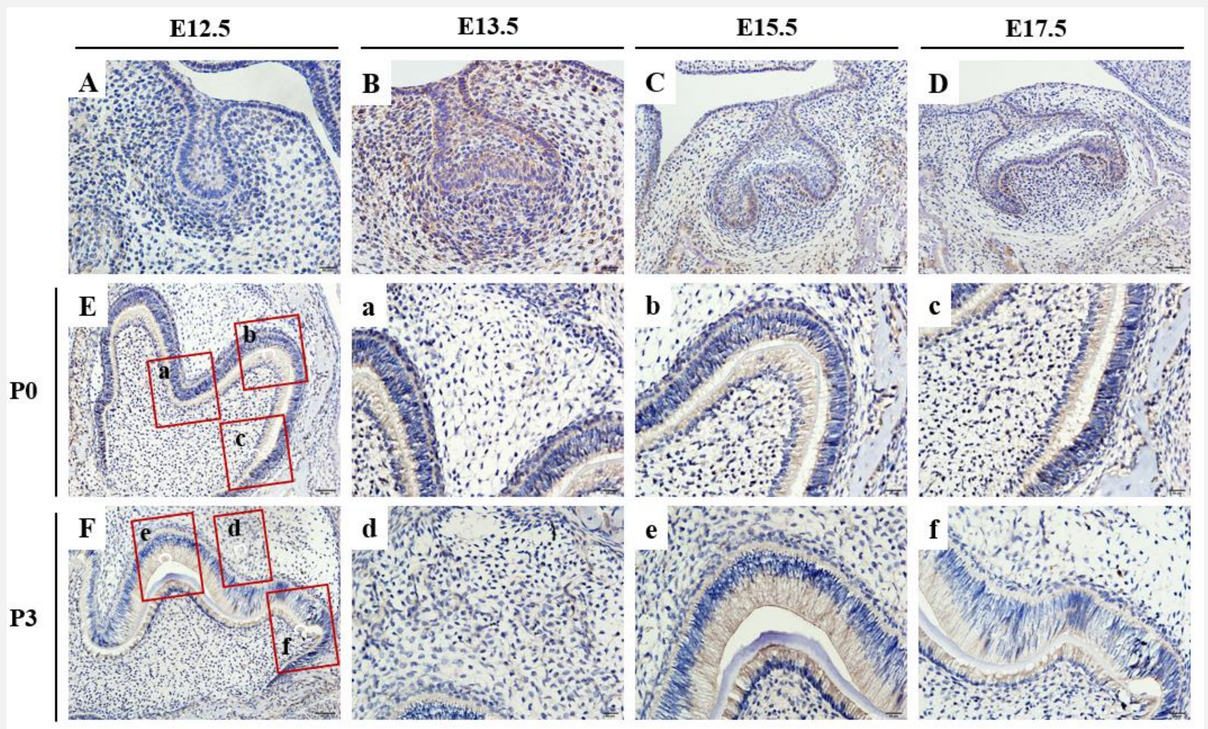

Fig2. Lin28b, the paralog of Lin28, was not specifically detected during tooth development. As Lin28b contains nuclear localization signal and nucleolus localization signal, it has been proved to present in the nucleus. The staining in Fig2 mainly present in the cytoplasm, considering not as Lin28b.

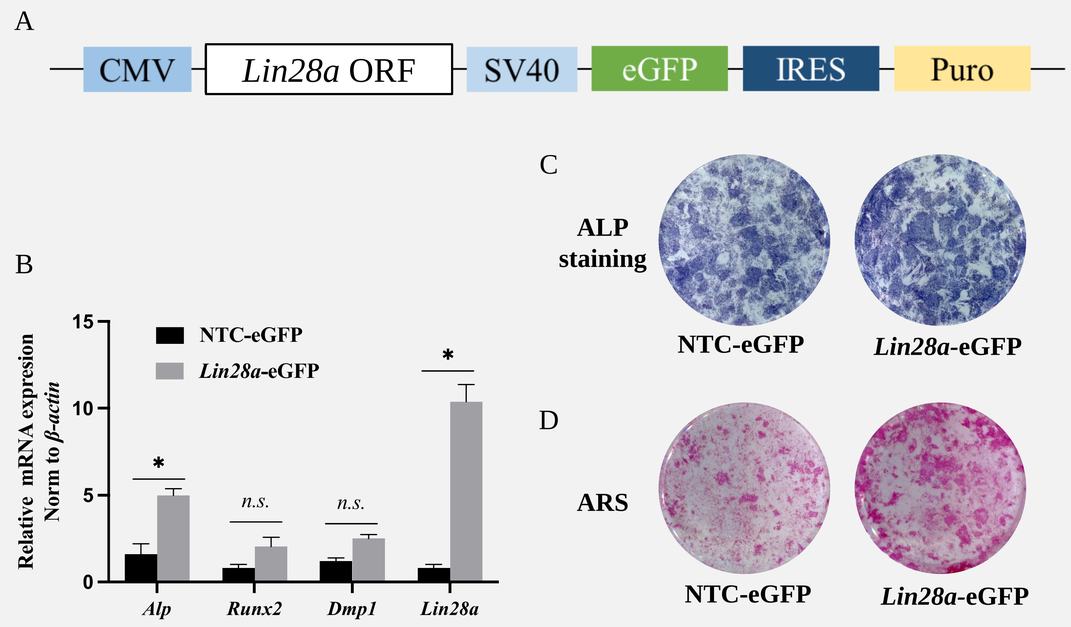

Fig3. A. Design and construct Lin28 overexpression lentivirus (*Lin28a*-eGFP). B. During odontogenic differentiation of dental papilla cells *in vitro,* the cells were infected with NTC-eGFP or *Lin28a*-eGFP. QPCR results proved that *Lin28* expression level elevation resulted in *Alp* expression increase. C~D. ALP staining and Alizarin red staining were both enhanced when *Lin28* expression level boosted.

Conclusions

The results revealed Lin28's potential role in promoting dental papilla cells' cell fate determination towards odontoblasts.

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