Abstract

Gli1^{+} cells in periodontal ligament contribute to

tooth socket healing

2022
Graduate School of Dentistry,
Health Sciences University of Hokkaido

Saki Fujii

【Purpose】

During the tissue repair process after tooth extraction, osteoblasts appear in the tooth socket and form alveolar bone. However, the source of these osteoblasts is still uncertain. Gli1, a downstream factor of Sonic hedgehog signaling, have been shown to exhibit stem cell characteristics in the periodontal ligament (PDL) of completed teeth (Shalehin N et al., J Dent Res, in press). Also, in mouse parietal bone, it has been reported that $Gli1^+$ cells are confined to the periosteum of the suture and when bone defects are created, $Gli1^+$ cells proliferate and differentiate into osteocytes in the bone regeneration area (Zhao H et al, Nat Cell Biol, 2015). In this study, we investigated the localization of $Gli1^+$ cells and their progeny cells after tooth extraction using Gli1-Cre^{ERT2};Rosa26-loxP-stop-loxP-tdTomato (iGli1/Tomato) mice. Furthermore, we evaluated the effect on extraction socket repair in the absence of $Gli1^+$ cells using Gli1-Cre^{ERT2}/Rosa26-loxP-stop-loxP-tdDTA (iGli1/DTA) mice, in which $Gli1^+$ cells are depleted after tamoxifen administration.

【Materials and Methods】

At the age of 4 weeks, iGli1/Tomato mice and iGli1/DTA mice were intraperitoneally injected with tamoxifen for 2 days, their maxillary second molars were extracted. Maxillary bones were removed and chemically fixed with 4 % paraformaldehyde before and 1, 3, and 7 days after tooth extraction. Some specimens were decalcified and embedded in paraffin, and serial sections of 4 μm thickness were prepared. Other specimens were freeze-embedded at -80°C, and un-decalcified frozen sections with a thickness of 5 μm were prepared. H-E staining and immunohistochemical staining (Osterix, Osteopontin, PCNA) were performed on paraffin sections. Non-decalcified frozen sections were stained for BMP4, Smad4, Runx2, Osterix, and Periostin, and their localization with Gli1/$Tomato^+$ cells was observed by confocal laser microscopy. In addition, calcein was administered intraperitoneally to some mice at 1, 3, 5, and 7days after tooth extraction, and newly-formed bone was labeled green. Moreover, we extracted the maxillary second molars of iGli1/Tomato mice and iGli1/DTA mice, and the amount of newly-formed bone in the tooth socket was compared.

【Results】

After 2 days of tamoxifen administration to iGli1/Tomato mice without tooth extraction, the PDL was positive for Periostin and Gli1/Tomato$^+$ cells were barely detected in the PDL. However, there are no Gli1/Tomato$^+$ cells on the surface of alveolar bone and bone marrow. At 1 day after tooth extraction, the PDL-like connective tissue was found on the surface of alveolar bone around tooth socket. Gli1/Tomato$^+$ cells were observed in the PDL-like tissue. At 3 days, although these inflammatory cells were disappeared, numerous Gli1/Tomato$^+$ cells harboring proliferating cell nuclear antigen were found in the tooth socket. Osteoblast differentiation marker Osterix$^+$ cells were observed sporadically at the margins of the tooth socket. After 7 days, Osteopontin$^+$ bone matrix was formed in the tooth socket apart from the original alveolar bone. Many Osterix$^+$ osteoblasts were arranged on the surface of this newly-formed bone, and a lot of Gli1/Tomato$^+$ cells were observed in the tooth socket, some of which were localized on the surface and inside the Calcein$^+$ newly-formed bone. Some of these Gli1/Tomato$^+$ cells expressing BMP4, Smad4, and Osterix on the surface of newly-formed bone. Comparison of the amount of newly-formed bone in iGli1/Tomato mice and iGli1/DTA mice 7 days after tooth extraction revealed that the amount of newly-formed bone in iGli1/DTA mice was significantly lower.

【Conclusion】

In this study, Gli1/Tomato$^+$ cells were found in PDL-like tissue that remained after tooth extraction, and were found to proliferate after tooth extraction. Some of Gli1$^+$ progenitor cells expressed Smad4, which activates BMP signaling, suggesting that they differentiated into osteoblast lineage cells via BMP signaling. Subsequently, Gli1/Tomato$^+$ cells differentiated to Runx2$^+$ and Osterix$^+$ osteoblasts and formed bone matrix in the tooth socket apart from the original alveolar bone. On the other hand, Gli1/Tomato$^+$ cells were barely detected in the marrow of the alveolar bone and did not proliferate after tooth extraction. Furthermore, depletion of Gli1/Tomato$^+$ cells in iGli1/DTA mice decreased the amount of newly-formed bone. These results suggest that Gli1$^+$ cells in the PDL proliferate after tooth extraction and contribute to alveolar bone formation in the tooth socket. The differentiation of Gli1$^+$ cells in the PDL is at least partly mediated by the BMP signaling pathway.