Abstract

Differentiation ability of Gli1^{+} cells in the periodontal ligament during orthodontic tooth movement

2022
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【Introduction】

Orthodontic force-induced osteogenic differentiation and bone formation at tension side play a pivotal role in orthodontic tooth movement (OTM). When mechanical stress is applied to the tooth and its periodontal tissues during treatment, osteoclasts that appear on the alveolar bone of the compression side undergo alveolar bone resorption. In contrast, in the periodontal ligament (PDL) of the tension side, mesenchymal stem cells (MSCs) differentiate into osteoblasts and bone matrix is formed on the original alveolar bone. The PDL is a fiber-reinforced tissue that contains an abundant vascular network and heterogeneous cell populations. This tissue transmits mechanical stress and maintain periodontal homeostasis. Thus, force-induced mechanical stress promotes MSC differentiation into osteoblasts. MSCs in the PDL are thought to differentiate into osteoblasts, cementoblasts, and fibroblasts. However, the precise localization of MSCs in the PDL as well as the origin of the osteoblasts during OTM remains unclear.

Gli1 is an essential transcription factor for hedgehog signaling and functions in undifferentiated cells during embryogenesis.

In this study, we examined the differentiation of $Gli1^{+}$-PDL cells during OTM to clarify the source of osteoblasts in the PDL side using a lineage-tracing analysis. The process of osteoblast differentiation in the progeny of $Gli1^{+}$ cells was evaluated immunohistochemically using osterix- and type I collagen-specific antibodies. The progenies of $Gli1^{+}$ cells on the newly formed bone matrix were also followed histologically by bone labeling in undecalcified frozen sections.

【Material and Methods】

Following the third tamoxifen injection to 8-week-old iGli1/Tomato mice for 2 days (10 mg/day), for medially moving the first molar, a nickel–titanium closed-coil spring (TOMY SEIKO) was tied to the upper anterior alveolar bone and left first molar using stainless steel (0.1 mm diameter; Unique Medical), under anesthesia. The samples were scanned with a Micro CT (inspeXio SMX-225CT, Shimadzu) at a voxel size of 9 μm. MY VGL software (Ratoc System Engineering) was used to generate three-dimensional (3D) reconstruction images of the maxilla. The distance between the first and second molars

was measured using the software. The samples were fixed with 4% paraformaldehyde at 4ºC for 12 h and Some samples were decalcified with 10% ethylenediaminetetraacetic acid (pH 7.4) at 4ºC for 14 days. After dehydration, the samples were embedded in paraffin and sagittally sectioned at a thickness of 4 μm. Other samples were embedded in 5% carboxymethyl cellulose gel (SECTION-LAB) at −80ºC without decalcification. Each frozen sample was sectioned with the film (Cryofilm Type IIc, SECTION-LAB) at a thickness of 5 μm. To visualize newly formed alveolar bone, iGli1/Tomato mice were administered calcein green (10 mg/kg body weight; DO-jindo) every other day during OTM.

【Results】

In the untreated tooth, ALP activity associated with bone mineralization was detected in the PDL around the mesial alveolar bone surface, but not around the distal surface. In contrast, cathepsin K^+ osteoclasts were localized to the alveolar bone on the distal side of the untreated tooth, suggesting that the first molar moves distally under physiological conditions. Gli1/Tomato$^+$ cells were rarely observed near endomucin$^+$ blood vessels in the PDL.

Two days after OTM initiation, the first molar moved medially. The number of Gli1/Tomato$^+$ cells increased along with numerous PCNA$^+$ cells in the PDL of the tension side. As some Gli1/Tomato$^+$ cells exhibited positive expression of osterix, an osteoblast differentiation marker, Gli1$^+$ cells probably differentiated into osteoblast progenitor cells. On day 5, the number of Smad$^+$ and β-catenin$^+$ cells were increased in the distal PDL On day 10, ALP activity was detected a strong response around the alveolar bone of the distal side. In addition, the newly formed bone labeled by calcein administration during OTM was detected on the surface of the original alveolar bone. Gli1/Tomato$^+$ cells expressing osterix localized to the surface and within the matrix of the newly formed bone.

In contrast, on days 2 and 10, most Gli1/Tomato$^+$ cells expressed type I collagen in the central part of the mesial PDL.

【Discussion】

In the untreated tooth, ALP activity was detected in the PDL around the mesial alveolar bone surface. In contrast, cathepsin K^+ osteoclasts were localized to the alveolar bone on the distal side. On day 10, however, ALP activity was detected only around the alveolar bone of the distal side and cathepsin K^+ cells were located on the alveolar bone of the mesial side. These results indicate that the direction of tooth movement of the first molar changed from distal to medial as a result of orthodontic force.

In this study, ALP activity was enhanced in the distal PDL, which resulted in bone formation. During this process, $Gli1^+$ cells proliferated from 2 days after OTM initiation and the number of progeny cells increased in the PDL. After OTM initiation, BMP and Wnt signalings are shown to be activated in the PDL of distal side. In addition, this activation was observed along with the proliferation and differentiation of $Gli1^+$ cells. On day 10, as the progeny cells of $Gli1^+$ cells expressed osterix and were distributed on the surface of the alveolar bone, $Gli1^+$ cells differentiated into osteoblasts in response to mechanical force.

In contrast, alveolar bone resorption occurred in cathepsin K^+ osteoclasts on the mesial side during OTM initiation. As osteoclasts derive from hematopoietic stem cells, Gli1/$Tomato^+$ cells in the PDL did not differentiate into osteoclasts and were not distributed on the alveolar bone of the mesial side. in the PDL of the mesial side, Gli1/$Tomato^+$ cells proliferated before day 10 and expressed type I collagen, suggesting that $Gli1^+$ cells differentiate into fibroblasts.

【Conclusion】

$Gli1^+$ cells in the PDL differentiate into osteoblasts, osteocytes, and fibroblasts during OTM. In addition, BMP and Wnt signalings may be involved in osteoblast differentiation of MSCs during this process. Our results indicate that MSCs expressing Gli1 may play an important role in OTM by supplying cells for bone remodeling as well as PDL reconstruction.