Abstract

The periodontal ligament contains stem cells that can differentiate into osteoblasts, cementoblasts, and fibroblasts. However, the characteristics and distribution of these cells remain uncertain. Gli1, an essential hedgehog signaling transcription factor, functions in undifferentiated cells during embryogenesis. Therefore, in the present study, to characterize the undifferentiated cells in the periodontal ligament, the localization pattern and the differentiation ability of Gli1^{+} cells were examined using a lineage-tracing system.

Gli1-Cre^{ERT2}/ROSA26-loxP-stop-loxP-tdTomato (iGli1/Tomato) mice were generated and administrated Tamoxifen for 2 days at 4 and 8 weeks of age. At 0–28 days after the final administration, the distribution of Gli1/Tomato^{+} cells in periodontal tissues was determined. In 4-week-old mice, Gli1/Tomato^{+} cells were barely detected in the periodontal ligament, around Endomucin-expressing blood vessels. These cells had proliferated over time, localizing in the periodontal ligament as well as on the bone and cementum surfaces for 28 days. However, in 8-week-old mice, Gli1/Tomato^{+} cells were quiescent, as evidenced by the fact that most cells did not show immunoreactivity for Ki-67.

Next, Gli1/Tomato^{+} and Gli1/Tomato^{-} cells were harvested from the periodontal ligament of 8-week-old iGli1/Tomato mice. To analyze whether Gli1^{+} cells have clonogenic and multilineage potentials, these cells were subjected to CFU-F and differentiation assays for osteoblasts, chondrocytes, and adipocytes. Gli1/Tomato^{+} cells in the periodontal ligament exhibited high CFU-F activity and were capable of osteogenic, chondrogenic, and adipogenic differentiation *in vitro*. In contrast, Gli1/Tomato^{-} cells did not show differentiation abilities.

Lastly, to observe the differentiation ability of Gli1^{+} cells during alveolar bone regeneration, the first maxillary molars of iGli1/Tomato mice, which had received Tamoxifen for 2 days, were extracted and transplanted into the hypodermis of wild-type mice. After 5 and 28 days, the teeth were excised with surrounding connective tissues and processed histologically. At 5 days after transplantation, the tooth root was surrounded by connective tissue and Gli1/Tomato^{+} cells were observed only near the tooth root and exhibited Osterix- and Ki67-immunoreactivity. At 28 days, the alveolar bone had been regenerated apart from the tooth root. Tomato fluorescence indicating progeny of Gli1^{+} cells was detected in the osteoblasts and osteocytes of the regenerated bone.

Our results suggest that Gli1^{+} periodontal ligament cells are identified as mesenchymal

stem cells with self-renewal ability and trilineage differentiation potential. Also, these cells contribute to the formation of periodontal tissue and can regenerate alveolar bone.