Glil-positive periodontal ligament cells possess stem cell properties and contribute to alveolar bone regeneration.

様式第10号（第５条関係）

2020年　2月　3日

学位論文審査並びに最終試験結果報告書

大学院歯学研究科長　　殿

主査　荒川　俊哉　㊞
副査　安彦　善裕　㊞
副査　村田　勝　㊞

今般 Nazmus Shalehin にかかわる学位論文審査並びに最終試験を行い下記の結果を得たので報告する。

記

1　学位論文題目　Glil-positive periodontal ligament cells possess stem cell properties and contribute to alveolar bone regeneration

2　論文要旨　別添

3　学位論文審査の要旨　別添（様式第12号）

4　最終試験の要旨　別添（様式第13号）

以上の結果 Nazmus Shalehin は博士（歯学）の学位を授与する資格の（ある）・ない　ものと判定する。

様式第12号（第５条・第13条関係）

学位論文審査の要旨

主査　荒川　俊哉

副査　安彦　善裕

副査　村田　勝

氏　　名 Nazmus Shalehin

学位論文題目 Gli1-positive periodontal ligament cells possess stem cell properties and contribute to alveolar bone regeneration

以下本文（15行目から1000字以内）

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

Gli1-CreERT2/ROSA26-loxP-stop-loxP-tdTomato (iGli1/Tomato) mice were generated and administrated Tamoxifen for two days at four and eight weeks of age. At 0–28 days after the final administration, the distribution of Gli1/Tomato+cells in periodontal tissues was determined. In four-week-old mice, Gli1/Tomato+cellswere barely detected in the periodontal ligament around the Endomucin-expressing blood vessels. These cells proliferated over time, localizing in the periodontal ligament, as well as on the bone and cementum surfaces for 28 days. However, in eight-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-ells were harvested from the periodontal ligament of eight-week-old iGli1/Tomato mice. To analyze whether Gli1+cells had clonogenic and multilineage potentials, the cells were subjected to colony-forming unit fibroblast (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 vitroÄi0. In contrast, Gli1/Tomato–cells did not show differentiation abilities.*

Finally, 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 two days, were extracted and transplanted into the hypodermis of wild type mice. After 5 and 28 days, the teeth were excised with the surrounding connective tissues and processed histologically. Five days post-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 the 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 a self-renewal ability and trilineage differentiation potential, contribute to the formation of periodontal tissue, and can regenerate alveolar bone.

様式第13号（第５条・第13条関係）

最終試験（学力の確認）の要旨

主査　荒川　俊哉　㊞
副査　安彦　善裕　㊞
副査　村田　勝　㊞

氏　　名　Nazmus Shalehin

以下本文（10行目から200字以内）

In this thesis, Nazumus Shalehin visualized the stem cells in PDL as Gli1 positive cells using iGli1/Tomato transgenic mouse and showed that the Gli1 positive stem cells had an ability of multi differentiation *in vitro* such as osteoblasts and chondrocytes. All data were shown well, and a manuscript is well done. He also adequately answered all questions. We recertified that this paper was valuable as thesis for the Ph.D. and he passed the final defense for Ph.D.