Journal of Oral Biosciences 60 (2018) 21–30

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Original article

Donor–host tissue interaction in allogenic transplanted tooth germ with special reference to periodontal tissue

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ARTICLE INFO

Article history:
Received 7 December 2017
Received in revised form
5 February 2018
Accepted 6 February 2018
Available online 19 February 2018

Keywords:
Bromodeoxyuridine
Cell differentiation
Periodontium
Transplantation
Mice (Transgenic)

ABSTRACT

Objectives: Limited biological evidence exists regarding donor–host interaction in the periodontal tissue during allogenic tooth germ transplantation. This study aimed to clarify donor–host tissue interactions during periodontal tissue healing following tooth germ transplantation.
Methods: This study compared the localization of putative stem cells in the periodontal ligament (PDL) by 5-bromo-2′-deoxyuridine (BrdU), Gli1, and periostin immunoreactions using pulse-chase paradigm (BrdU prenatal labeling: peritoneal pulse injections at embryonic days [E] 15–17) in TetOP–H2B–GFP mice (doxycycline administration at E14.5). The current study characterized periodontal tissue healing following allogenic tooth grafts in GFP-labeled donor or host and wild-type mice by pulse-chase paradigm and GFP, BrdU, Gli1, and periostin immunohistochemistry.
Results: BrdU prenatal labeling demonstrated that dense label-retaining cells (BrdU–LRCs) disappeared from the PDL by postnatal week 2 (P2W). However, H2B–GFP–LRCs were localized in the PDL of TetOP–H2B–GFP mice during P3–8W, and Gli1-positive cells in the PDL increased at P2–3W, showing that H2B–GFP–LRCs in the PDL are derived from non-proliferating cells during E15–17. Transplanted molars formed cusps and roots and erupted into occlusion by two weeks postoperatively. The junctional epithelium and tooth-related zone of PDL were exclusively composed of donor cells, whereas the PDL alveolar-related zone was a hybrid structure of donor and host cells.
Conclusions: The current tooth germ transplantation suggests that the PDL contains putative stem cells, which never proliferate during E15–17, and is composed of resident dental follicle-derived cells and other cell population.

1. Introduction

The prevalence of non-syndromic tooth agenesis varies from 2.2% to 10.1% for the world population, excluding third molars, the most commonly missing teeth [1]. Because dental implants are contraindicated in younger patients with missing teeth during jaw development, autologous tooth germ transplantation of third molars is an available method for tooth replacement instead of dental implants to harmonize their occlusion with jaw development. Allogenic tooth transplantation is an alternative treatment for replacing a missing tooth, even in adult patients where a suitable donor tooth is not available in case of autogenic transplants. Recently, we developed an experimental mice model of allogenic tooth germ transplantation [2]. The dynamic donor–host interaction during transplant development affects the characteristics of the dental pulp in the transplants. However, there is little biological evidence regarding the donor–host interaction in the periodontal tissue following tooth germ transplantation, despite the paramount importance of this tissue in transplantation success. In case of allogenic tooth transplantation in Crlj:CD1 (ICR) mice, the periodontal tissue recovers, even in case of immunological rejection [3]. This indicates that host cells replace donor cells even after immunological rejection, leading to the fact that clinical application of allogenic tooth transplantation could be achieved.

The periodontal ligament (PDL) consists of osteoblasts, osteoclasts, fibroblasts, epithelial cell rests of Malassez, immune cells, undifferentiated mesenchymal cells, stem cells, and cementoblasts

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http://dx.doi.org/10.1016/j.job.2018.02.002
1349-0079/

[4]. Pluripotent stem cells are present in the PDL [5–7] and the dental follicle (DF) [8], are derived from neural crest cells, and exhibit generic mesenchymal stem cell-like properties, including expression of marker genes and differentiation into mesenchymal cell lineages (osteoblasts, chondrocytes, and adipocytes) *in vitro* and, to some extent, *in vivo* [5–7]. After tooth extraction, PDL stem cells can be obtained from the alveolar bone [9,10]. Following human PDL stem cell transplantation into periodontal defects in immunocompromised mice, PDL-like tissue is regenerated, suggesting their involvement in alveolar bone regeneration [6]. Because the regenerative capacity and plasticity of the PDL are largely dependent on PDL stem cells, understanding of the mechanisms that regulate the maintenance of PDL stem cells and their differentiation capacity may be clinically applied in autogenic and allogenic tooth germ transplantation. However, there are no available data as to where PDL stem cells are localized in the *in vivo* periodontal tissue.

Recent evidence that adult stem cells are primarily responsible for tissue healing and regeneration has demonstrated that two types of stem cells—active (in the cell cycle) and quiescent (out of the cell cycle in a lower metabolic state)—exist in renewal tissues, including the intestinal crypt, bone marrow, and hair follicle [11]. Adult stem cells in the dental pulp and PDL are quiescent stem cells that can actively proliferate only under the pathological condition of tooth and its supporting tissue injury. Recently, we succeeded in identifying slow-cycling long-term label-retaining cells (LRCs) in the dental pulp or dental pulp stem/progenitor cells by prenatal labeling methods, in which the thymidine analog 5-bromo-2′-deoxyuridine (BrdU) is administered into embryonic Wistar rats and ICR mice [12,13]. These methods clarified that dense BrdU–LRCs are localized in the center of the dental pulp associated with blood vessels and these cells differentiate into odontoblast-like cells. However, these methods failed to identify BrdU–LRCs in the PDL. To overcome the inherent issue of the BrdU labeling method, including its inability to label truly quiescent stem cells, we used doxycycline-inducible histone 2B–green fluorescent protein (H2B–GFP) transgenic mice [14].

Allogenic tooth germ transplantation in GFP and wild-type (WT) mice demonstrated that donor-derived GFP reactions were maintained in the pulp cells, including spindle-shaped mesenchymal cells, odontoblasts, pericytes, and endothelial cells, and that host-derived cells immigrated into the dental pulp postoperatively [2]. These findings suggested that pulpal mesenchymal stem cells are exclusively derived from inherent pulp cells and prenatally immigrated non-inherent pulp cells. Although it is assumed that the PDL is composed of DF-derived cells and other cell population, the origin of PDL cells is yet to be clarified. Allogenic tooth germ transplantation with GFP and WT mice could mimic the contribution of DF-derived and non-DF-derived cells during odontogenesis. Thus, this study aimed at investigating donor–host interaction during tooth germ transplantation using prenatal BrdU labeling in H2B–GFP and GFP mice to clarify the relationship between resident PDL cells and immigrating cells during odontogenesis.

2. Materials and methods

2.1. BrdU labeling and tooth germ transplantation protocol

WT C57BL/6J [B6] mice were obtained from the Charles River Laboratories of Japan (Yokohama, Japan). Three intraperitoneal injections of BrdU (150 mg/kg) were administered to WT mice (once a day at embryonic days [E] 15–17) via the pregnant mother according to the prenatal BrdU labeling method for mice [12]. The detailed procedures for tooth germ transplantation are described previously [2] (Fig. 1).

2.2. Tooth germ transplantation in GFP transgenic and ICR mice

The mandibular first molar tooth germ (1–2-days-old) of GFP transgenic mice [15] or WT mice was transplanted into the alveolar socket of the maxillary M1 (12–14-days-old) of WT or GFP transgenic mice, respectively, in addition to tooth germ transplantation (1–2-days-old) in ICR mice (Charles River Laboratories of Japan).

2.3. TetOP–H2B–GFP mice

TetOP–H2B–GFP mice [B6; 129S4-Gt(ROSA)26Sor < tm1(rtTA*M2) Jae > Colla1 < tm7(tetO-HIST1H2BJ/GFP)Jae > /J] were purchased from Jackson Laboratories [14]. For transgene expression, doxycycline

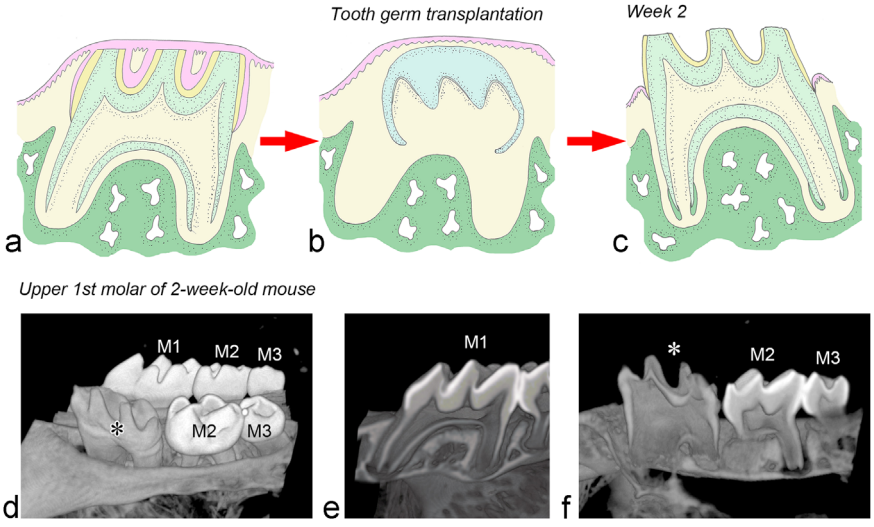

Fig. 1. A scheme indicating the tooth germ transplantation procedure (a–c) and micro-CT images (d–f) of the transplant and contralateral tooth on Day 14. (a–c) The tooth germ was allografted in the alveolar socket of a 2-week-old mouse (b) where the upper right first molar was extracted after creating a mucous membrane flap (a). The transplanted molar formed cusps and roots and erupted into occlusion by two weeks postoperatively (c). (d) The three-dimensionally reconstructed transplant (*) showing the normal configuration consisting of six cusps and two roots. (e) Sagittally viewed contralateral tooth with thick enamel and dentin. (f) Sagittally viewed transplant (*) representing the poor formation of enamel. M1, first molar; M2, second molar; M3, third molar.

Table 1
Number of animals in each experiment (labeled, TetOP-GFP, ICR, donor labeled, donor GFP, or host GFP mice) for statistical analyses of chronological changes of positive cells (BrdU- or Gli1-positive cells.

	P1	P3	P5	P7(-P9)	P14	P18	P3W	P8W	Total
Labeled ICR mice (odontogenesis)	4	4	3	3	3		3		20
TetOP-H2B-GFP mice (odontogenesis)							3	3	6
ICR mice (transplantation)		2		1	3				6
Donor labeled B6 mice (transplantation)		4	5	5	7				21
Donor GFP mice (transplantation)						1			1
Host GFP mice (transplantation)							2		2

(Sigma D9891, 2 mg/ml, supplemented with sucrose at 50 mg/ml) was added to drinking water at E14.5.

2.4. Tissue preparation

Three intraperitoneal injections (once per day: E15–17) of BrdU (150 mg/kg body weight) were administered to pregnant ICR mice to map BrdU–LRCs in the mature tissues of postnatal animals. For histological analyses, including immunohistochemistry, ICR mice and/or TetOP–H2B–GFP mice at postnatal Days 1 (P1) ($n = 4$), P3 ($n = 4$), P5 ($n = 3$), P7 ($n = 3$), and P14 ($n = 3$) and Week 3 ($n = 3$) and 8 ($n = 3$) were used in this experiment (Table 1). Materials for tooth germ transplantation were collected from groups of 1–7 non-labeled host ICR and B6 mice and groups of 1–2 paired GFP and WT mice (Table 1). At each stage, the animals were transcardially perfused with physiological saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) under deep anesthesia by an intraperitoneal injection of chloral hydrate (350 mg/kg). Analysis by micro-CT (Elescan; Nittetsu Elex, Tokyo, Japan) was performed to examine the morphological differences between transplants and control teeth at 14 days after transplantation before the decalcification of samples. Following decalcification in 10% EDTA 2Na solution for three weeks at 4 °C, the specimens were embedded in paraffin, and sagittal sections of 5 μm were prepared. The paraffin sections were mounted on glass slides and stained with hematoxylin and eosin (H&E).

2.5. Immunohistochemical analysis

For BrdU-labeling assays, the sections were processed for Calbiochem BrdU Immunohistochemistry System (EMD Biosciences, Darmstadt, Germany; catalog number: HCS30). BrdU immunohistochemistry was performed as described elsewhere [12]. Immunohistochemistry for Gli1, periostin, and GFP was conducted using rabbit anti-Gli1 polyclonal antibody diluted to 1:500 (Novus Biologicals, Littleton, CO, USA; catalog number: NBP1–78259), rabbit anti-periostin polyclonal antibody diluted to 1:5000 (Abcam, Cambridge, UK; catalog number: ab14041), and rabbit anti-GFP polyclonal antibody diluted to 1:1000 (Medical & Biological Laboratories Co., Nagoya, Japan; catalog number: 598). The Envision horseradish peroxidase (HRP) kit (Dako Japan; catalog number: K5027) and avidin–biotin peroxidase complex (ABC) (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA) kit were used in addition to biotinylated anti-rat IgG (Vector Laboratories; catalog number: BA-4000) diluted to 1:100 and biotinylated anti-rabbit IgG (Vector Laboratory; BA-1000) diluted to 1:200 antibodies.

2.6. Statistical analysis for BrdU- and Gli1-positive cells

The number of dense and granular BrdU-, H2B–GFP-, and Gli1-positive cells in the PDL (grid [16.5×10^3 μm^2], [2.7×10^3 μm^2], or [$2.9–4.9 \times 10^3$ μm^2], respectively) was calculated in the developing and transplanted teeth. We distinguished dense and granular BrdU–LRCs according to previously described criteria [2]. In brief, in dense BrdU–LRCs cells, the nucleus is densely stained (arrows), whereas granular cells were other labeled cells. Data were obtained from the samples of developing (P1 [$n = 4$], P3 [$n = 4$], P5 [$n = 3$], P7 [$n = 3$], P14 [$n = 3$], and P21 [$n = 3$] or Weeks 3 [$n = 3$] and 8 [$n = 3$] weeks postnatal) and transplanted teeth (3 [$n = 3–4$], 7 [$n = 3–5$], and 14 [$n = 3–7$] days postoperatively). Furthermore, the number or proportion of cells among different time points in the transplanted teeth was compared using one-way analysis of variance (ANOVA) for multiple comparisons, adjusted by Bonferroni's test, or Student's *t*-test using SPSS 16.0 J or 20.0 J for Windows (SPSS Japan, Tokyo, Japan).

3. Results

3.1. Tooth development and tooth germ transplantation in ICR mice

The postnatal developmental process of maxillary first molars was as follows: dentinogenesis and amelogenesis had begun by Day 3 (Fig. 2a), crown morphogenesis was complete on Day 5 (Fig. 2c), root formation was initiated and enamel maturation was almost complete on Day 7 (Fig. 2e), and root formation continued to reach the stage when tooth eruption would start soon on Day 14 (Fig. 2g). The transplants were maintained in the alveolar sockets of host animals without immunological rejection until Day 9 (Fig. 2b, d, f). Amelogenesis was disturbed because of mechanical injury during the operation. Immunological rejection occurred in the dental pulp, and accumulation of inflammatory cells and sparse connective tissue without odontoblasts was observed, whereas the PDL showed normal features (Fig. 2h).

3.2. BrdU–LRCs and Gli1- and periostin-positive cells during tooth development and healing after tooth germ transplantation in B6 mice

Dense and granular BrdU–LRCs were observed in the DF and PDL until Week 1, and subsequently, dense BrdU–LRCs decreased significantly ($P < 0.01$) and almost disappeared in the PDL on Week 2 (Fig. 3a, d, g, j, m, and p and 5a). Gli1-positive cells were recognized in the DF and PDL until Week 1, and thereafter, the numbers increased significantly and occupied the PDL (Fig. 3b, e, h, k, n, and q and 5b). The DF and PDL along with the periosteum showed intense periostin-positive reactions (Fig. 3c, f, i, l, o, r). Three-dimensionally reconstructed transplants on Day 14, demonstrated by micro-CT analysis, showed normal configuration consisting of six cusps and two roots and the completion of tooth eruption and occlusion (Fig. 1d). Sagittally viewed transplants represented poor formation of enamel compared with the maxillary second (M2) and third molars (M3) and the contralateral maxillary first molar (Fig. 1e, f). The transplants were maintained in the alveolar sockets of host animals without immunological rejection throughout the examination period (Fig. 4a, d, g). Root formation progressed normally, and odontoblasts were arranged throughout

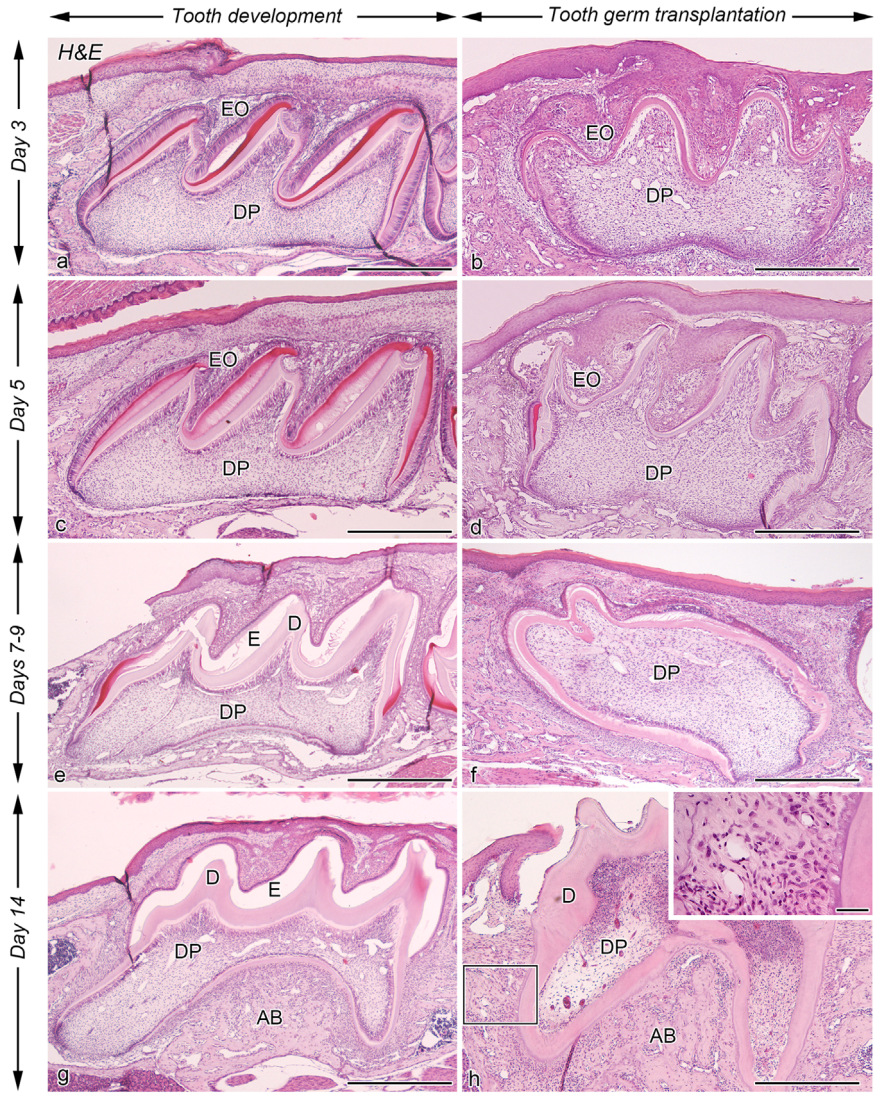

Fig. 2. H&E staining of the developing teeth (a, c, e, and g) and transplants (b, d, f, and h) on 3 (a, b), 5 (c, d), 7–9 (e, f), and 14 (g, h) days postnatal or postoperatively. (a, c, e, and g) Dentinogenesis and amelogenesis started by Day 3, crown morphogenesis was complete on Day 5, root formation started and most of the enamel maturation was complete on Day 7, and root formation continued to reach the stage when tooth eruption would start soon on Day 14. (b, d, f, and h) The transplants were maintained in the alveolar sockets of host animals without immunological rejection until Day 9. Amelogenesis was disturbed because of mechanical injury during the operation. Immunological rejection occurred in the dental pulp where accumulation of inflammatory cells and sparse connective tissue without odontoblasts was observed, whereas the periodontal ligament (inset) showed normal features. (Inset) Higher magnified view of the boxed area in h. AB, alveolar bone; D, dentin; DP, dental pulp; E, enamel space; EO, enamel organ. Bars, 500 μm (a–h), 50 μm (inset).

the periphery of the pulp tissue. The chronological changes in dense BrdU–LRCs and Gli1-positive cells in the DF and PDL were almost the same as those during normal tooth development, i.e., dense BrdU–LRCs decreased in number and almost disappeared in the PDL (Fig. 4b, e, and h and 5c) and Gli1-positive cells increased significantly in the PDL at Week 2 (Fig. 4c, f, and i and 5d). Periostin immunoreactivity was consistently observed in the DF and PDL during the experimental periods, and periostin-positive cells appeared in the dental pulp and increased with healing of the transplants (Fig. 4j–r).

3.3. Contribution of host and donor cells to various cells in transplanted teeth

In tooth germ transplantation in GFP transgenic mice as the donor and WT as the host, GFP-positive cells constituted the dental pulp of the transplant and the tooth-related zone of the PDL, except for immigrated mesenchymal cells wherein blood vessels were composed of GFP-positive and -negative endothelial cells (Fig. 6a, b). The junctional epithelium (JE) exclusively showed a GFP-positive reaction, whereas the gingival epithelium never represented a positive reaction (Fig. 6c). In tooth germ transplantation using GFP transgenic mice as the host and WT as the donor, GFP-positive vascular elements and immigrated cells were observed, most of which showed a dendritic appearance, in the dental pulp (Fig. 6d). In contrast, GFP-positive cells were localized in the alveolus-related zone of the PDL, and few GFP-positive cells were observed in tooth-related zone (Fig. 6d–f).

3.4. Chronological changes in H2B–GFP–LRCs in the PDL during tooth development in TetOP–H2B–GFP mice

Dense and granular (weak) H2B–GFP–LRCs were consistently observed in the PDL during the examination period (Fig. 7). Dense LRCs occupied 12%–18% of the PDL and were primarily distributed in the tooth-related zone.

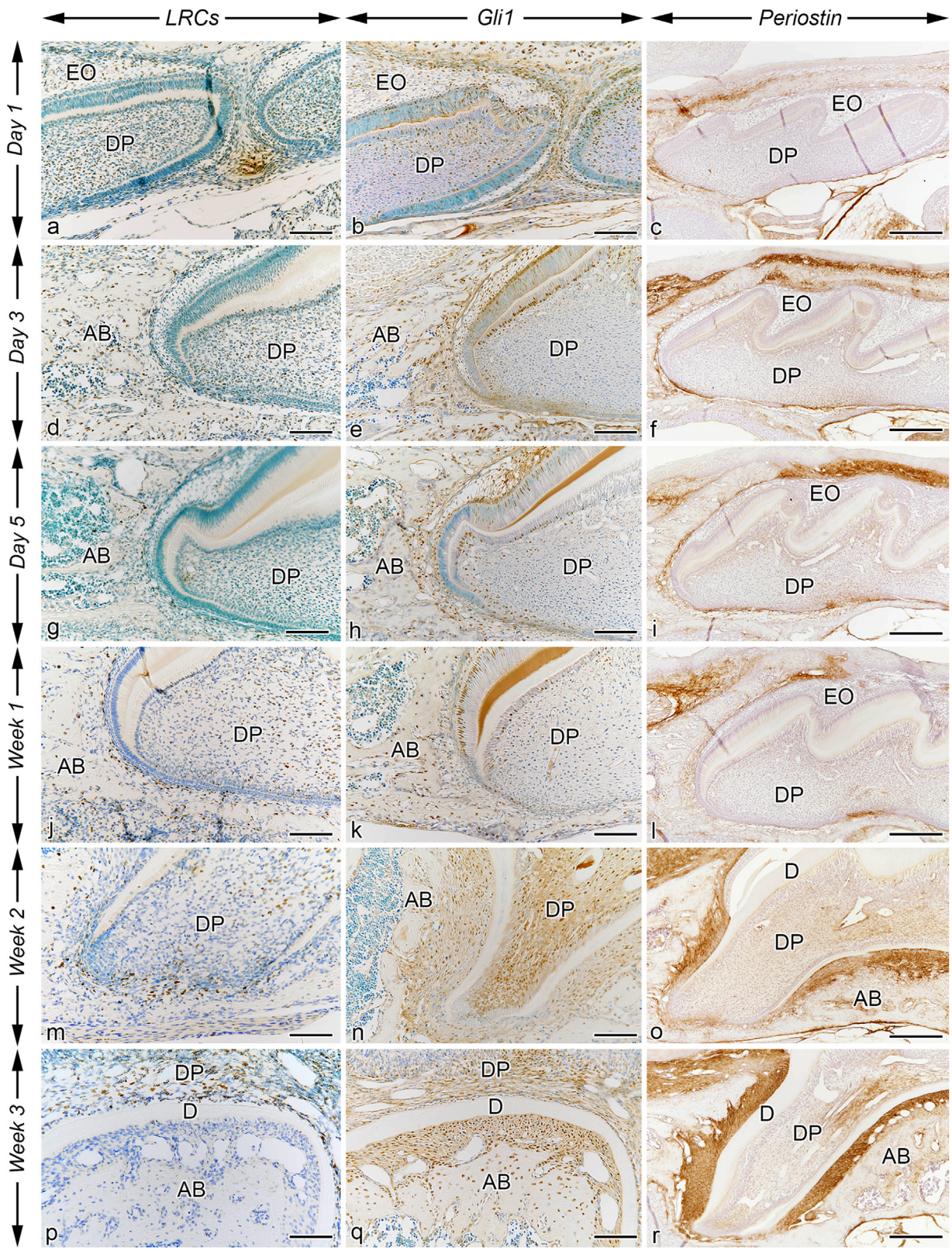

Fig. 3. BrdU (a, d, g, j, m, and p), Gli1 (b, e, h, k, n, and q), and periostin immunoreactivities (c, f, i, l, o, and r) in the developing teeth on Day 1 (a–c), Day 3 (d–f), Day 5 (g–i), Week 1 (j–l), Week 2 (m–o), and Week 3 (p–r). (a, d, g, j, m, and p) Dense and granular BrdU-label-retaining cells (LRCs) are observed in the dental follicle (DF) and periodontal ligament (PDL) until Week 1, and, subsequently, dense BrdU–LRCs decrease in number and almost disappear in the PDL by Week 2. (b, e, h, k, n, and q) Gli1-positive cells are recognized in the DF and PDL until Week 1, and, thereafter, the cells occupied the PDL. (c, f, i, l, o, and r). The DF and PDL in addition to the periosteum show intense periostin-positive reactions. AB, alveolar bone; D, dentin; DP, dental pulp; EO, enamel organ. Bars, 250 μm (c, f, i, l, o, and r), 100 μm (a, b, d, e, g, h, j, k, m, n, p, and q).

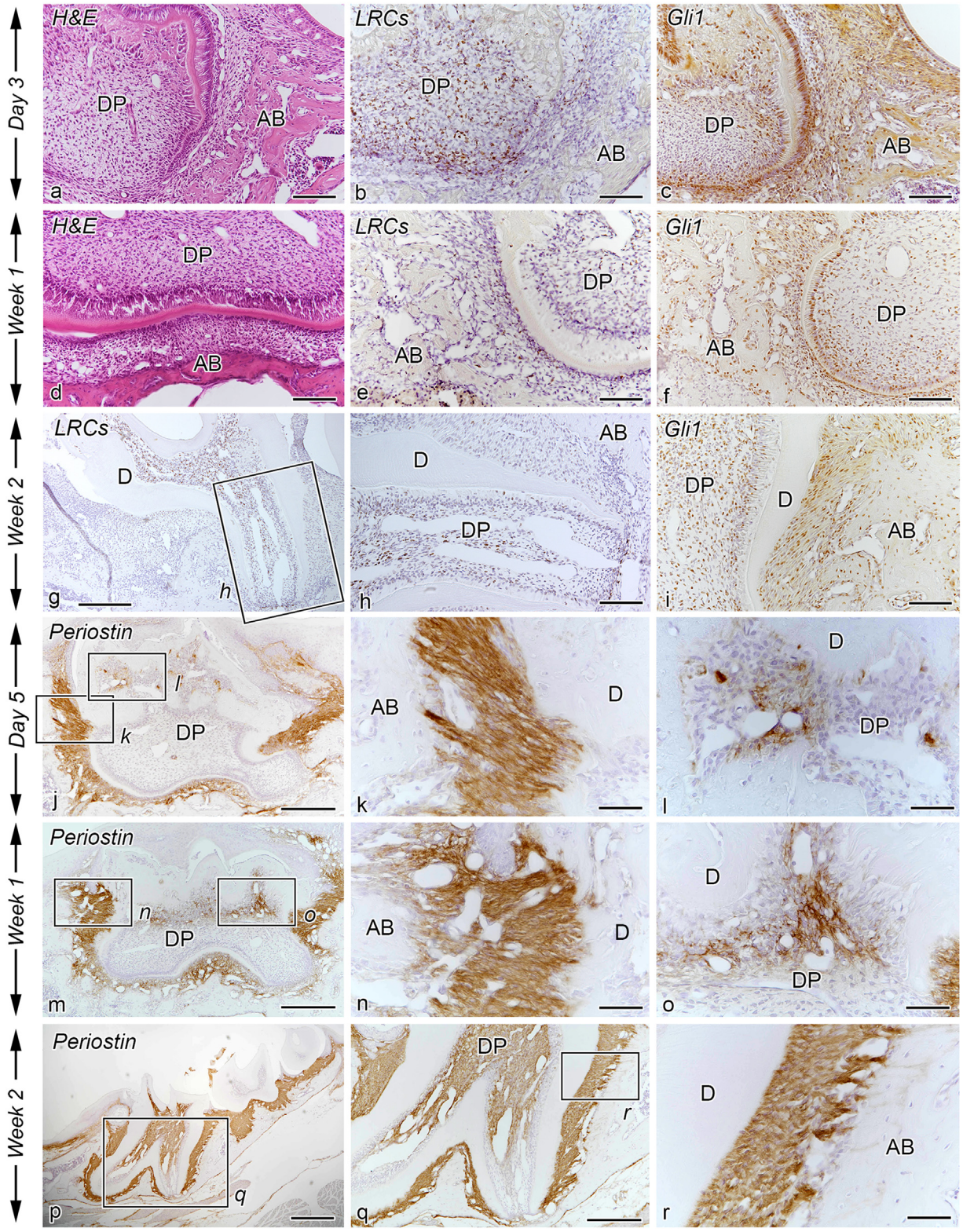

Fig. 4. H&E staining (a, d) and BrdU (b, e, g, and h), Gli1 (c, f, and i), and periostin immunoreactivities (j–r) in the transplants on Day 3 (a–c), 5 (j–l), Week 1 (d–f, m–o), and Week 2 (g–i, p–r). (a–i) Transplants are maintained in the alveolar sockets of host animals without immunological rejection throughout the examination period. Root formation progresses normally, and odontoblasts are arranged throughout the periphery of the pulp tissue. Dense BrdU-label-retaining cells (LRCs) decrease and almost disappear in the PDL, and Gli1-positive cells increase in the PDL at Week 2. Figure h is the higher magnified view of the boxed area in g. (j–r) Periostin immunoreactivity is consistently observed in the DF and PDL during the experimental period, and periostin-positive cells appear in the dental pulp and increase in number with healing of the transplants. Figures k, l, n, o, and q are the higher magnified views of the boxed areas in j, j, m, m, and p, respectively. AB, alveolar bone; D, dentin; DP, dental pulp. Bars, 500 μm (p), 250 μm (g, j, m, and q), 100 μm (a–f, h, and i), 50 μm (k, l, n, o, and r).

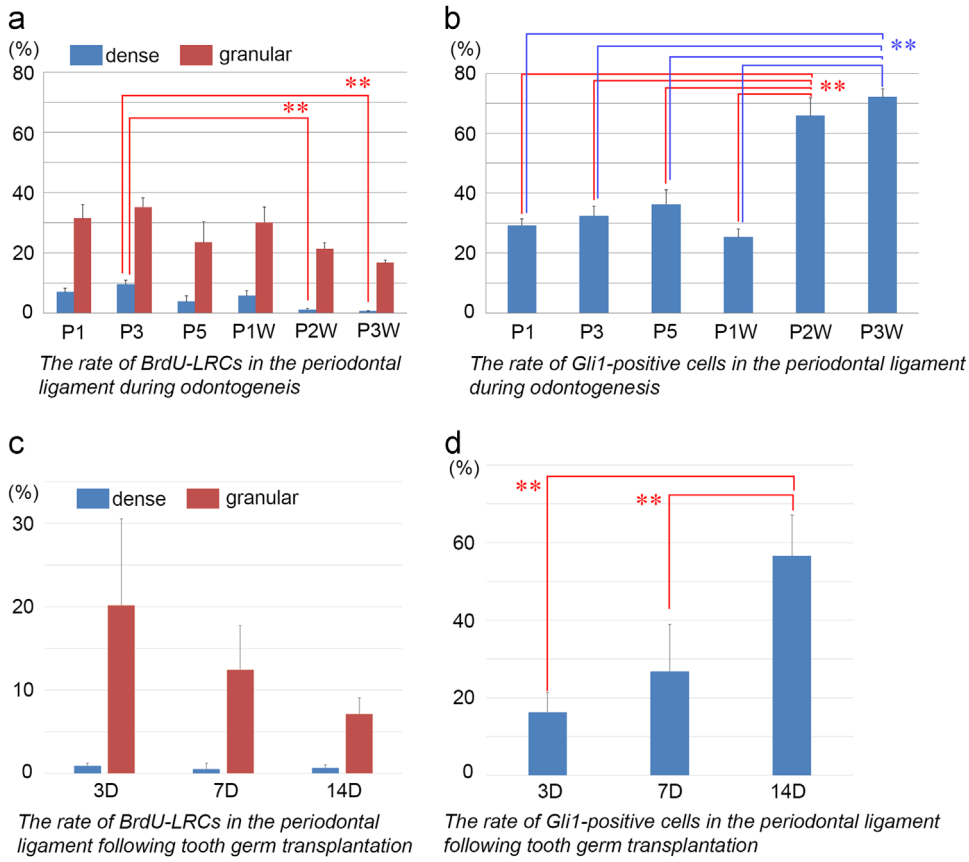

Fig. 5. Chronological changes in the proportion of BrdU-label-retaining cells (LRCs) (a, c) and Gli1-positive cells (b, d) to pulpal cells (%) in the developing teeth (a, b) and transplants (c, d). (a) Dense BrdU–LRCs decrease significantly and almost disappear in the PDL at Week 2. (b) Gli1-positive cells increase significantly at Week 2. (c) Dense BrdU–LRCs decrease and almost disappear in the PDL at Week 2. (d) Gli1-positive cells significantly increase in the PDL at Week 2.

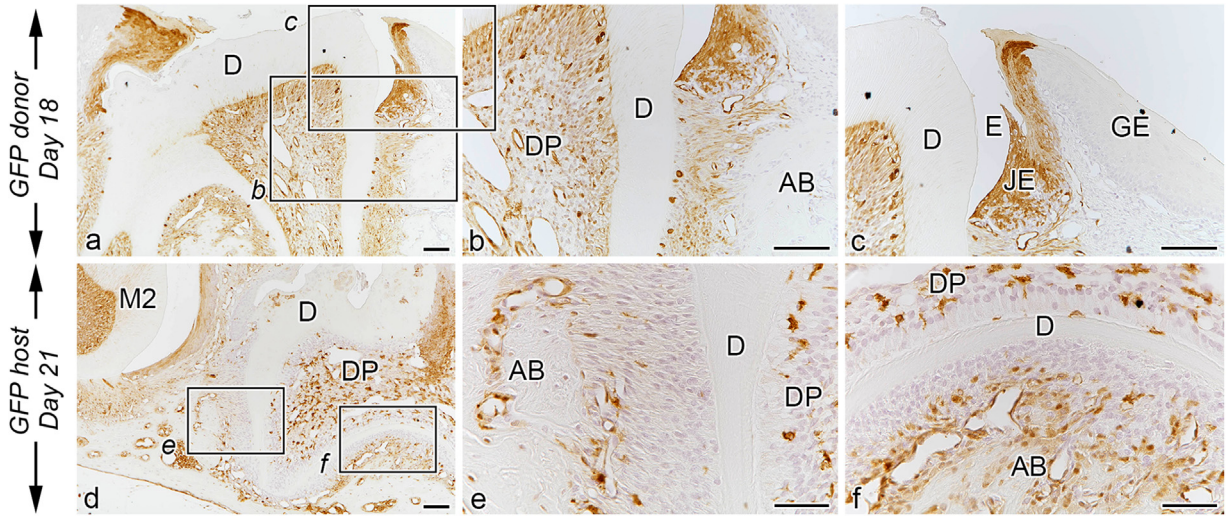

Fig. 6. GFP-immunoreactivity in GFP transgenic (a–c) and wild-type (WT) transplants (d–f) in the WT (a–c) and GFP transgenic recipients (d–f) on Days 18 (a–c) and 21 (d–f). (a, b) GFP-positive cells constitute the dental pulp of the transplant and the tooth-related zone of the PDL, except for immigrated mesenchymal cells, in which blood vessels are composed of GFP-positive and -negative endothelial cells. (c) The junctional epithelium (JE) exclusively showed a GFP-positive reaction, whereas the gingival epithelium (GE) never represented a positive reaction. (d–f) GFP-positive vascular elements and immigrated cells are observed, most of which showed a dendritic appearance, in the dental pulp. GFP-positive cells are localized in the alveolus-related zone of the PDL, and few GFP-positive cells are observed in the tooth-related zone. Figures b, c, e, and f are the higher magnified views of the boxed areas in a, a, d, and d, respectively. AB, alveolar bone; D, dentin; DP, dental pulp; M2, second molar. Bars, 100 μm (a–d), 50 μm (e, f).

4. Discussion

4.1. Immunological rejection after tooth germ transplantation

The present tooth germ transplantation in ICR mice demonstrated that immunological rejection may occur in the dental pulp. In contrast, the periodontal tissue showed normal features in the transplants, suggesting that donor-derived periodontal tissue cells were replaced with host-derived cells after immunological attack by cytotoxic T cells. Tooth transplantation in GFP mice in this study demonstrated that most donor-derived cells were localized in the tooth-related zone of the PDL. In case of immunological rejection,

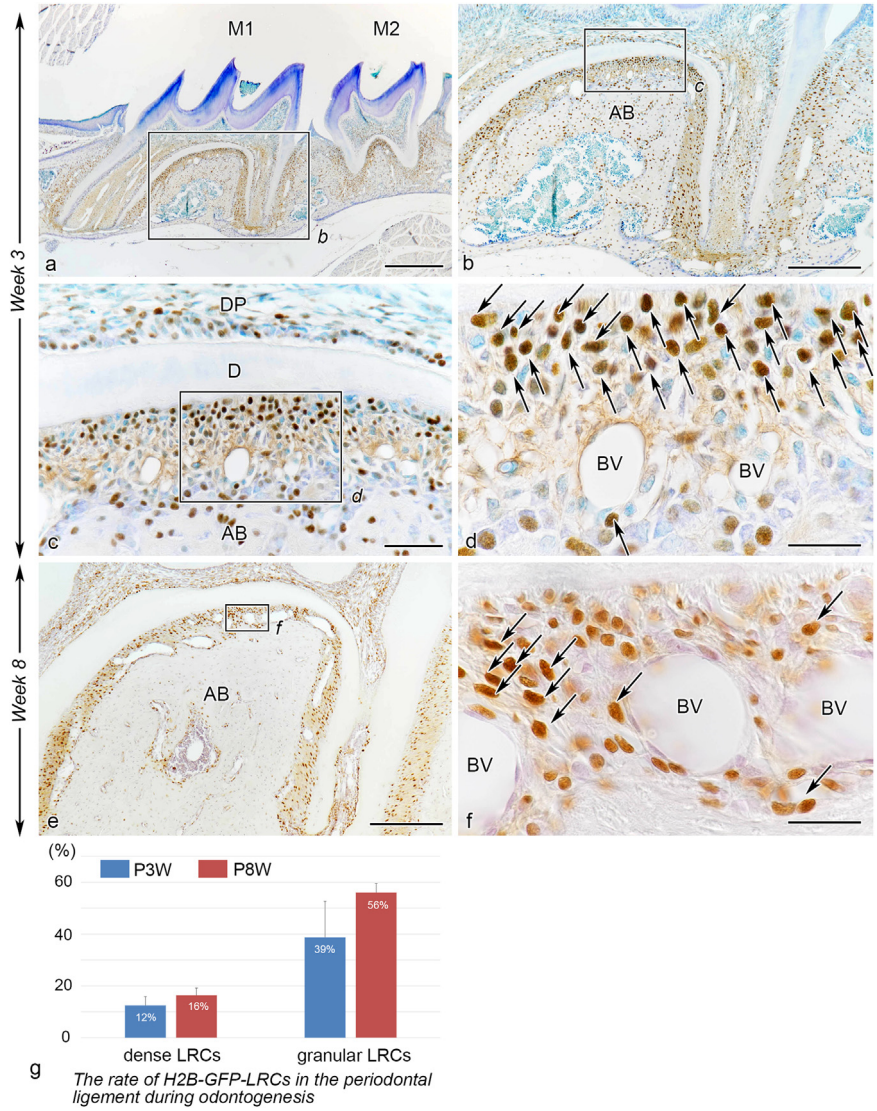

Fig. 7. GFP-immunoreactivity in the developing teeth of the TetOP–H2B–GFP mice at Weeks 3 (a–d) and 8 (e, f) and the chronological changes in the proportion of H2B–GFP–label-retaining cells (LRCs) to pulpal cells (%) in the developing tooth. (a–f) Dense and granular (weak) H2B–GFP–LRCs are consistently observed in the PDL during the examination period. Dense LRCs (arrows) occupy 12%–18% of the PDL and are primarily distributed in the tooth-related zone. (g) There is no significant difference between Weeks 3 and 8 with respect to the number of dense and granular (week) H2B–GFP–LRCs. Figures b, c, d, and f are the higher magnified views of the boxed areas in a, b, c, and e, respectively. AB, alveolar bone; D, dentin; DP, dental pulp; M1, first molar; M2, second molar; M3, third molar. Bars, 500 μm (a), 250 μm (b, e), 50 μm (c), 25 μm (d, f).

most cells residing in the tooth-related zone disappeared, including resident adult stem cells, causing the PDL to be different from the original tissue. The alveolar-related zone of the PDL in the transplants primarily included other cell population derived from the host tissue. Further studies are necessary to elucidate the PDL characteristics in allogenic transplants with immunological rejection.

4.2. LRCs in the PDL

The present analysis of LRCs in the PDL during molar tooth development in TetOP–H2B–GFP mice demonstrated that dense H2B–GFP–LRCs occupied 12%–18% of the PDL, even 3–8 weeks postnatal. Because LRCs are quiescent adult stem cells [16], their distribution in the PDL may be related to its high plasticity [17]. Prenatal BrdU labeling by three intraperitoneal injections of BrdU (once a day at E15–17) failed to detect BrdU–LRCs in the PDL, indicating that cell populations lacking proliferative activity during E15-17 constitute the PDL at Week 3. In contrast, BrdU–LRCs were maintained in the dental pulp, even at Week 3 [12], suggesting that pulpal stem/progenitor cells are totally different from stem/progenitor cells in the PDL in terms of their origin. The coincidence of the distribution patterns of H2B–GFP–LRCs and Gli1-immunoreactivity in the PDL at Week 3 is similar to the co-localization of BrdU–LRCs and Gli1-positive cells in the dental pulp [16], although the number of H2B–GFP–LRCs were lower than that of Gli1-positive cells. Thus, it is reasonable that quiescent stem cells in the PDL and in other tissues of neurogenesis, mammary gland development, angiogenesis, hair morphogenesis, and dental pulp development are regulated by Shh signaling [16,18–21], although Shh signaling may also induce differentiation of the PDL [22]. Thus, the reason behind the increase in Gli1-positive cells in the PDL at P2–3W remains to be elucidated.

4.3. Host–donor interaction in the PDL of transplanted teeth

The current allogenic tooth germ transplantation in GFP mice demonstrated the origin of PDL cells, i.e., the relationship between DF-derived and non-DF-derived cells, as this experiment mimics normal tooth development. DF-derived cells (donor cells) were

primarily localized in the tooth-related zone, whereas most non-DF-derived cells (host cells) were in the alveolus-related zone. Judging from the evidence that H2B–GFP–LRCs are primarily distributed in the tooth-related zone, it is probable that the contribution of non-DF-derived cells as LRCs is low. However, because the present study did not perform allogenic tooth germ transplantation in TetOP–H2B–GFP mice, further studies using this experimental model are needed to elucidate the behavior of the two subpopulations.

The present study using GFP mice also clarified that the JE, an epithelial component directly attached to the tooth surface, originates from donor enamel organ cells. These findings are in accordance with the results of a recent study using a bioengineered tooth [23]. This topic has not been scientifically elucidated for a long time, despite the notion that the JE is derived from the reduced enamel epithelium of the tooth germ, which has been widely described in text books [4]. Furthermore, the reduced enamel epithelium-derived JE is maintained lifetime without replacement, but the maintenance of the oral epithelium remains controversial [24,25]. Further experiments, using natural tooth germ transplantation derived from WT and GFP mice into GFP and WT mice, respectively, are needed to clarify whether the odontogenic epithelium is maintained in the mature JE or not.

Immunohistochemistry experiments for periostin demonstrated that periostin-positive cells appeared in the dental pulp by Day 5, increased, and were distributed throughout the pulp tissue, except for the odontoblast layer and apical portions, until Week 2 in the transplanted tooth germ. Because periostin is specifically and strongly expressed in human and mouse PDL tissues and that periostin expression is strongly induced during PDL cell differentiation [26], it is reasonable that PDL cells migrate into the pulp chamber during transplanted tooth germ development. Interestingly, periostin-positive cells appeared in the pulp chamber, even in the process of natural tooth development, in this study; however, the intensity of the immunoreaction was considerably low, suggesting that PDL cells may migrate into the pulp tissue or pulp cells may transform their phenotype into the PDL type. The same phenomenon is also observed in case of allogenic tooth transplantation [27].

5. Conclusions

The JE and the tooth-related zone of the PDL were exclusively composed of donor cells, whereas the alveolar-related zone of the PDL was a hybrid structure of donor and host cells. H2B–GFP–LRCs were localized in the PDL in TetOP–H2B–GFP mice during P3–8W, and Gli1-positive cells increased in the PDL at P2–3W. Thus, H2B–GFP–LRCs or putative stem cells in the PDL are derived from non-proliferating cells during E15–17 because three intraperitoneal injections of BrdU (once a day at E15–17) failed to detect BrdU–LRCs in the PDL. The current tooth germ transplantation suggests that the PDL contains putative stem cells, which never proliferate during E15–17, and is composed of resident DF-derived cells and other cell population.

Acknowledgement

We are grateful to Prof. Masaru Okabe for providing GFP mice. This work was supported by Grants-in-Aid for Scientific Research (B) (no. 17H04366 to HO) and (C) (nos. 23593026 and 17H11953 to KN-O) from JSPS.

Ethical approval

All animal experiments were conducted in compliance with the protocol reviewed by the Institutional Animal Care and Use Committee and approved by the President of Niigata University (Permit Number: Niigata Univ. Res.132–2).

Conflicts of interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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