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

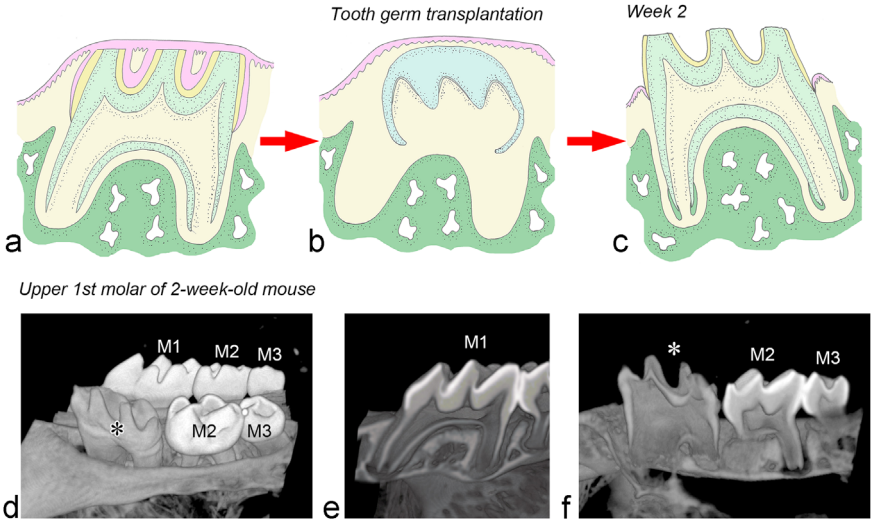

Fig. 1. A scheme indicating the tooth germ transplantation procedure (a–c) and micro-CT images (d–f) of the transplant and contralateral tooth on Day 14. (a–c) The tooth germ was allografted in the alveolar socket of a 2-week-old mouse (b) where the upper right first molar was extracted after creating 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.