Journal of Oral Biosciences ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Journal of Oral Biosciences

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

Review

Differentiation capacity and maintenance of dental pulp stem/progenitor cells in the process of pulpal healing following tooth injuries

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ARTICLE INFO

Article history:
Received 5 January 2017
Received in revised form
28 January 2017
Accepted 6 February 2017

Keywords:
Apoptosis
Dental pulp
Stem cells
Transplantation
Mice (ICR)

ABSTRACT

Background: Recently, we demonstrated that a pulse of 5-bromo-2'-deoxyuridine (BrdU) given to prenatal animals discloses the existence of slow-cycling long-term label-retaining cells (LRCs), or putative adult stem/progenitor cells, which reside in the dental pulp. Using several tooth injury models such as cavity preparation, tooth replantation, tooth or tooth crown transplantation, and tooth germ transplantation, we have clarified the dynamics and differentiation capacity of LRCs postoperatively. Our recent studies have demonstrated that allogenic tooth transplantation may influence the maintenance of dental pulp stem/progenitor cells.

Highlight: Dense LRCs are competent to proliferate and differentiate into odontoblast-like cells after tooth injuries. In the case of tooth replantation and autogenic tooth transplantation, dense LRCs remain in the perivascular environment in the center of the dental pulp for a long period. In contrast, allograft LRCs disappear from this niche during postoperative weeks 2–4. The loss of LRCs, even in cases without immunological rejection, is attributed to the extensive apoptosis taking place in these cells, with the exception of newly differentiated odontoblast-like cells.

Conclusion: Host and recipient interactions that occur with allografts disturb the maintenance of putative stem/progenitor cells, resulting in the disappearance of these cell types.

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1. Introduction

To elucidate the biological property of dental pulp stem cells (DPSCs), their localization *in vivo* has been one of the most important subjects in dental pulp biology. Stem cells possess the following marked characteristics: (1) stem cells maintain their undifferentiated state; (2) stem cells undergo unlimited cell division; and (3) a stem cell divides into one daughter cell that is identical to the original stem cell, and into another cell that gives

Abbreviations: (DPSCs), dental pulp stem cells; (BrdU), 5-bromo-2'-deoxyuridine; (LRCs), label-retaining cells

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http://dx.doi.org/10.1016/j.job.2017.03.001
1349-0079/

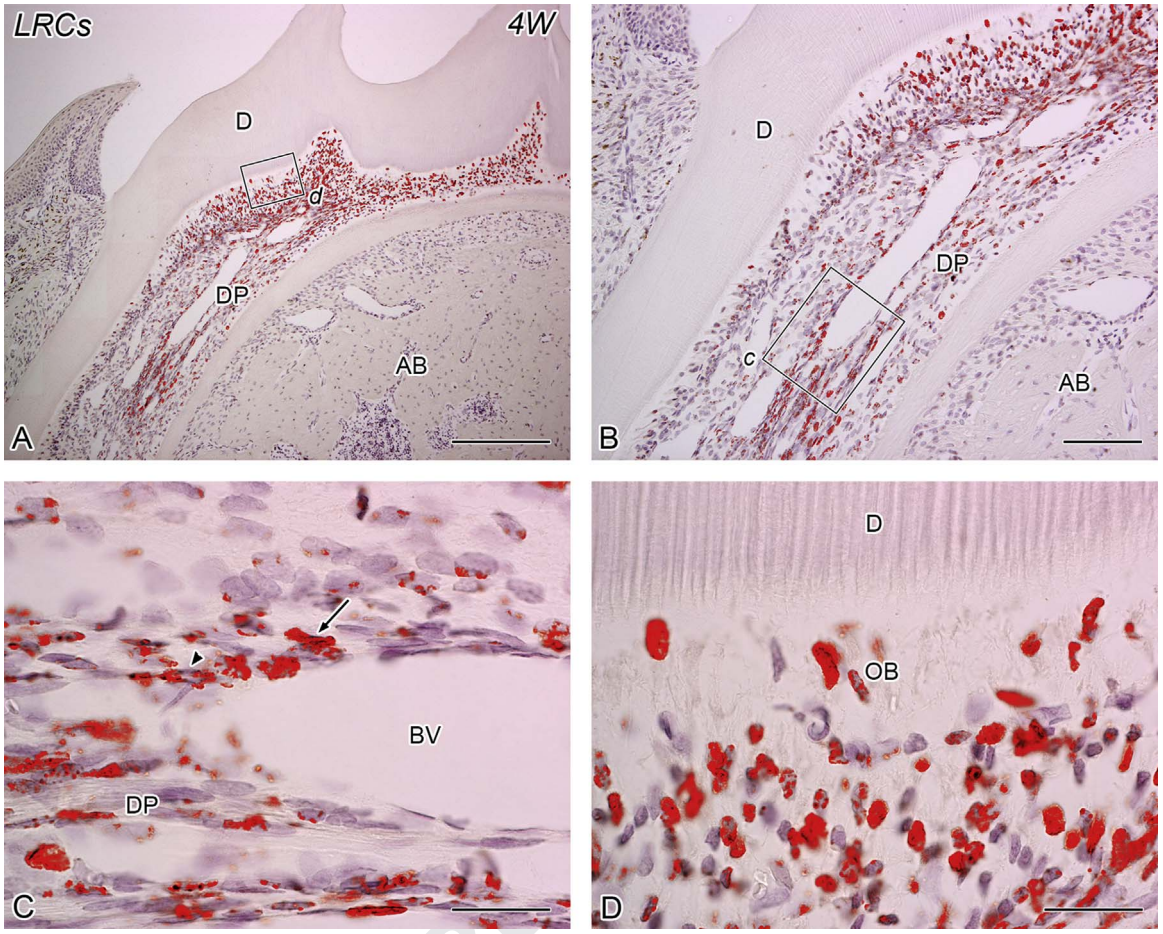

Fig. 1. 5-bromo-2'-deoxyuridine (BrdU)-immunoreactivity in the BrdU-labeled intact mouse tooth, 4 weeks after birth (*AB,* alveolar bone; *BV,* blood vessel; *D,* dentin; *DP,* dental pulp; *OB,* odontoblasts). The brown color in the BrdU-immunopositive cells changed to red. (**A, B**) Dental pulp contains numerous label-retaining cells (LRCs) with dense or granular reactions. (**C**) Higher magnification of the boxed area in **B**. Two types of LRCs are distinguished: those with dense (arrow) and granular (arrowhead) reactions. Dense LRCs are primarily associated with the blood vessels in the center of the dental pulp, whereas granular LRCs are distributed throughout the dental pulp. (**D**) Higher magnification of the boxed area in **A**. The coronal odontoblasts include many dense LRCs. Bars: 250 μm (**A**), 100 μm (**B**), 25 μm (**C, D**).

rise to transit amplifying cells [1]. Administering the thymidine analog 5-bromo-2'-deoxyuridine (BrdU) into young animals at an appropriate time could label putative stem/progenitor cells as slow-cycling long-term label-retaining cells (LRCs) [2,3]. During the chase period, these BrdU labels in the actively proliferating or transit amplifying cells are diluted out or lost due to multiple cell divisions, whereas the slow-cycling cells, including stem cells, retain the incorporated BrdU in their nucleus. Our recent studies utilizing the prenatal labeling method in embryonic Wister rats and ICR mice demonstrated that the incorporation of BrdU into cell division presumably allows adult stem/progenitor cells to be labeled as dense LRCs [4,5]. These dense LRCs reside in the perivascular niche in the center of the dental pulp, and express surface markers of mesenchymal stem cells, including STRO-1 and CD146, suggesting that dental pulp stem/progenitor cells can be identified as dense LRCs. However, the prenatal labeling method has some inherent problems. First, non-dividing quiescent cells cannot be labeled because BrdU incorporation requires cell division. Secondly, functional assays of LRCs isolated based on the intensity of labeling is not possible, since analyzing BrdU incorporation requires fixation [6]. Third, a number of studies suggest that BrdU is toxic to cells and animals [7,8]. Finally, differentiated odontoblasts are also densely labeled, as the timing of BrdU administration is consistent with the stage of odontoblast differentiation [4,5,9–11]. To date, however, no alternative universal method has been established for the identification of DPSCs *in vivo*, and the precise localization of these cells is still controversial.

Tertiary dentin and bone tissue can be formed in the dental pulp following tooth replantation or transplantation [12–19]. Our recent studies have shown that the healing pattern in the dental pulp is related to the maintenance of dense LRCs. Tertiary dentin formation is induced in the dental pulp in cases when dense LRCs remain present, whereas the pulp chamber is obturated by bone tissue in cases when dense LRCs are not maintained [5,9]. In allogenic tooth transplantation, however, the dense LRCs do not remain permanently in the dental pulp, and dentin formation continuously occurs to obliterate the pulp cavity, even in cases without immunological rejection [9]. This observation suggests that the maintenance of dense LRCs, including dental pulp stem/progenitor cells, plays a role in the homeostasis of the pulp tissue and inhibition of continuous dentin deposition. Furthermore, disappearance of LRCs in the center of the pulp cavity has been observed in another animal model of the allograft: tooth crown transplantation into the sublingual region. This experiment demonstrated that LRCs disappear in the pulp cavity due to extensive apoptosis occurring postoperatively at Week 2 [10]. Removal of the pulp floor may have a negative effect on the fate of dense LRCs in the pulp chamber following allogenic transplantation because most of the cells located in the center of the dental pulp are excluded from the pulp floor resection. Therefore, there is a need to establish a new experimental model for transplantation that avoids removing the cells residing in the center of the dental pulp.

In the case of tooth replantation, the pulp chamber is never obliterated (although reductions in pulpal size occur) compared

Please cite this article as: Saito K, Ohshima H. Differentiation capacity and maintenance of dental pulp stem/progenitor cells in the process of pulpal healing following tooth injuries. J Oral Biosci (2017), http://dx.doi.org/10.1016/j.job.2017.03.001