O40-RESPONSES OF BRDU-LABEL-RETAINING DENTAL PULP CELLS TO ALLOGENIC TOOTH TRANSPLANTATION INTO MOUSE MAXILLA.

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Key words
Bromodeoxyuridine, Cell differentiation, Dental pulp, Odontoblasts, Stem cells, Transplantation, Mice (Inbred ICR).

Introduction
Autogenic tooth transplantation is now a common procedure in dentistry for replacing a missing tooth. However, there are many difficulties in clinical application of allogenic tooth transplantation because of immunological rejection. Recently, we have established the successful experimental animal model using mice for allogenic tooth transplantation into the maxilla (Unno H *et al.*, 2009). Furthermore, our recent study has demonstrated that a pulse of the thymidine analog BrdU given to the prenatal animals revealed the existence of slow-cycling long-term label-retaining cells (LRCs), putative adult stem cells, reside in the pulp tissue (Ishikawa Y *et al.*, 2010). This study aims to clarify responses of BrdU-label-retaining dental pulp cells to allogenic tooth transplantation into mouse maxilla using *in situ* hybridization for osteopontin (OPN) and periostin and immunocytochemistry for BrdU, nestin, OPN, and periostin. Furthermore, the relationship between donor and host cells in the healing process has been analyzed using GFP mice.

Materials and Methods
Two to 3 peritoneal injections of BrdU were given to pregnant Crlj:CD1(ICR) mice to map dense LRCs in the mature tissues of born animals. The labeled born animals at 2 weeks after birth were used for tooth transplantation. The upper-right first molars (M1) of BrdU-labeled and non-labeled mice (2 weeks old) were extracted under anesthesia, and the extracted teeth were allografted in the original socket in the non-labeled and BrdU-labeled mice, respectively, after the extraction of M1. Materials were collected in groups of animals at intervals of 1, 3, 5, 7 days, 2, 4, and 8 weeks after allogenic tooth transplantation. Furthermore, the allogenic tooth transplantation was performed between GFP and non-GFP mice. The upper-left M1 of the same animal was used as control.

Results
In the control group, nestin-immunoreactivity was exclusively expressed in the odontoblasts, and numerous dense LRCs were mainly resided in the center of the dental pulp of BrdU-labeled animals, associating with blood vessels. Tooth transplantation caused degeneration of the odontoblast layer, resulting in the disappearance of nestin-positive reactions in the dental pulp. On postoperative Days 1–3, the pulp chamber was mainly occupied by inflammatory lesions including numerous neutrophils, fibrin networks, and a hemorrhage. On postoperative Days 5–7, tertiary dentin formation commenced next to the preexisting dentin where nestin-positive odontoblast-like cells were arranged in the successful cases. Three types of healing patterns were recognized until Day 14: tertiary dentin, the mixed form of dentin and bone-like tissue formation, and immunological rejection. In the case of BrdU-labeled transplanted teeth, dense LRCs were maintained in the center of the dental pulp beneath the newly differentiated odontoblast-like cells, whereas LRCs disappeared in the area beneath the bone-like tissue. On the other hand, LRCs were not recognized in the pulp chamber of non-labeled transplants through the experimental period. Interestingly, the periodontal tissue recovered even in the case of immunological rejection in which the pulp chamber was replaced by sparse connective tissue. In such cases, the donor periodontal tissue was replaced by the host tissue.

Conclusion
These results suggest that the maintenance of BrdU-label-retaining dental pulp cells is the decisive factor for the regeneration of odontoblast-like cells in the process of pulpal healing following tooth transplantation.

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