Cell Dynamics in the Process of Pulpal Healing Following Tooth Injuries

Hayato Ohshima

Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Key words: Dental Cavity Preparation, Dental Pulp, Histocompatibility Antigens Class II, Immunohistochemistry, Odontoblasts, Regeneration, Tooth Replantation

This study focuses on the cell dynamics of putative adult stem cells and the role of antigen-presenting cells (APC) in the pulp tissue following tooth injuries such as cavity preparation and tooth replantation. In molar teeth of 100-day-old rats, intense heal-shock protein (HSP)-25-immunoreactivity (IR) was found in the cell bodies of odontoblasts. Cavity preparation caused degeneration of the odontoblast layer resulting in the loss of HSP-25-IR in the injured dental pulp at the early stages after tooth injury. Numerous APC appeared along the pulp-dentin border and extended their cell processes into the dentinal tubules during 12-24 hours after cavity preparation, and weak HSP-25-IR appeared in the subodontoblastic layer after 24 hours. Newly differentiated odontoblast-like cells with HSP-25-IR were arranged at the pulp-dentin border, and the APC retreated towards the subodontoblastic layer by postoperative 3 days after tooth injury. Interestingly, proliferative activity appeared in the putative adult stem cells in the subodontoblastic layer at Day 2, and increased in number in the dental pulp during Days 3-5. Tooth replantation also induced similar cell dynamics in the dental pulp, although the appearance of the APC at the pulp-dentin border was delayed in the case of tooth replantation. Thus, common cellular events occurred during pulpal regeneration following two different experimental injuries. These findings indicate that the temporal appearance of the APC at the pulp-dentin border suggests their participation in odontoblast differentiation as well as in initial defense reactions during the pulpal regeneration process.

The dentin-pulp complex is capable of repair after tooth injuries such as dental caries, attrition, abrasion and restorative procedures including cavity preparation. The procedures of cavity preparation and tooth replantation induce destructive changes in odontoblasts at the affected site as well as an acute inflammatory reaction. If the odontoblasts survive, they are capable of depositing further reactionary dentin. If not, pulpal mesenchymal cells take the place of the degenerated odontoblasts to differentiate into odontoblast-like cells (OLC) resulting in the formation of reparative dentin. This phenomenon may indicate that adult stem cells exist in the pulp tissue of a matured tooth that has completed eruption and root formation.

Heat-shock protein (HSP)-25, the family of low molecular weight HSPs, is expressed in various normal cells as well as under stressful conditions, although it was first discovered under the latter conditions. Our recent studies have demonstrated a stage-specific expression pattern of HSP-25-IR in the odontoblasts under normal and experimental conditions, suggesting that this protein is a useful marker for the differentiation of odontoblasts during the pulpal healing process after tooth injury.

The dental pulp contains many immunocompetent cells that serve in an initial defense reaction and for antigen-presentation. The most abundant cells, having a class II major histocompatibility complex (MHC) antigen, are referred to as antigen-presenting cells (APC). APC have been reported to show characteristic reaction patterns under various experimental conditions such as tooth grinding, tooth replantation, and carious teeth. These cells are thought to have diverse functions in odontoblast differentiation in the rat dental pulp in addition to ordinary antigen presentation under experimental conditions. These findings therefore lead us to the possibility that APC play an important role in the process of pulpal healing after tooth injuries. However, the exact differentiation process of OLC is not clearly understood. The purpose of the present study is to clarify the cell dynamics of putative adult stem cells and the role of APC in the pulp tissue following tooth injuries such as cavity preparation and tooth replantation.

Wistar rats, 4 weeks and 100 days old, were used in this study for cavity preparation and tooth replantation, respectively. Materials were collected at intervals of 0-24 hours and 3-10 days after cavity preparation, and 1-5 days after replantation. At each stage, the animals were anesthetized, intraperitoneally injected with BrdU (150 mg/kg) two hours before the fixation and transcardially perfused with physiological saline followed with 4% paraformaldehyde in a 0.1 M phosphate buffer. The maxillae were removed en bloc and immersed in the same fixative. Following decalcification in a 5% ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) solution for 4 weeks at 4 °C, the tissue blocks were embedded in paraffin for paraffin sections, or processed for cryo-sections. For the immuno-peroxidase procedure, paraffin or frozen sections were processed for the avidin-biotin peroxidase complex (ABC) method using either anti-HSP-25- or OX6-antibody, or the BrdU immunohistochemistry system using anti-BrdU-antibody.

In the present immunocytochemical study, both cavity preparation and tooth replantation caused drastic time-related alterations in HSP-25 expression in the odontoblasts: the chronological changes in HSP-25-IR indicated the degeneration/regeneration process of the odontoblasts under pathological conditions. Furthermore, the APC dramatically changed their locations and populations in correlation with the degeneration/regeneration of the odontoblast layer.

In untreated control teeth, intense HSP-25-IR was found in the cell bodies of odontoblasts. Cavity preparation caused the degeneration of the odontoblast layer resulting in the loss of HSP-25-IR in the injured dental pulp at the early stages after tooth

Correspondence to Hayato Ohshima, Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Gakkocho-dori, Niigata 951-8514, Japan, Tel: +81-25-227-2812 fax +81-25-227-0804.
e-mail: histoman @dent. niigata-u.ac.jp

injury. Numerous APC appeared along the pulp-dentin border and extended their cell processes into the dentinal tubules during 12-24 hours after cavity preparation, and weak HSP-25-IR appeared in the subodontoblastic layer after 24 hours. Newly differentiated odontoblast-like cells (OLC) with HSP-25-IR were arranged at the pulp-dentin border, and the APC retreated towards the subodontoblastic layer by postoperative 3 days after tooth injury.

Tooth replantation also induces alterations in the expression of HSP-25-IR in the odontoblasts. However, the timing of disappearance/acquisition of HSP-25-IR in the dental pulp and the appearance of APC along the pulp-dentin border is different from that in the case of cavity preparation: the odontoblasts totally loose their immunoreaction for HSP-25 by 1 day and acquire it by 5 days after tooth injury, and APC appeared along the pulp-dentin border after 3 days. The time lag between two experimental studies may be attributed to the differences in the mode and extent of damage to the odontoblasts: cavity preparation injured the odontoblasts mechanically through their cell processes, whereas tooth replantation caused insufficient nutritional status by the interruption of the vascular supply to degenerate the pulpal cells. The dentin-pulp complex has the ability to respond to a variety of pathological conditions by localized deposition of a tertiary dentin matrix. In the pathological conditions that cause the death and destruction of odontoblasts, the damaged dentin is replaced by reparative dentin secreted by a new generation of OLC. The present results provide evidence that the dental pulp gives rise to new generations of OLC secreting reparative dentin in both experimental models such as cavity preparation and tooth replantation by monitoring the degeneration/regeneration process of HSP-25-positive cells. When odontoblasts undergo degeneration after tooth injury, mesenchymal cells of the pulp tissue differentiate into OLC that form tertiary dentin. Notch signaling, which is an evolutionally conserved mechanism controlling organ formation and morphogenesis, is an important element in the pulpal regeneration after tooth injury: Notch receptor is expressed in the cells of the subodontoblastic layer and ligands are expressed in odontoblasts. The mesenchymal cells showing weak HSP-25-IR apart from the pulp-dentin border at 24 hours after cavity preparation might be enhanced odontoblast precursors destined for terminally differentiating odontoblasts. It is noteworthy that proliferative activity appeared in the putative adult stem cells in the subodontoblastic layer at Day 2, and increased in number in the dental pulp during Days 3-5. These results indicate that the differentiation of progenitor cells into OLC and their arrangement along the pulp-dentin border occurred before the proliferation of the putative adult stem cells in the subodontoblastic layer.

An abundant distribution of APC has been reported in the dental pulp of rats under normal conditions. The APC participate in the initial recognition and the processing of antigenic substances resulting in antigen-presenting. Following exposure to antigens, they migrate to the lymphoid tissue via afferent lymph to activate T-cells and induce their cell proliferation. Interestingly, a temporal aggregation of APC appeared at the pulp-dentin border 12-24 hours after cavity preparation and 3 days after tooth replantation. This phenomenon may be explained by the idea that the pulpal APC respond actively to bacterial or noxious substances derived through the exposed dentinal tubules. In the case of tooth replantation, however, dentinal tubules are not exposed to the oral environment. Furthermore, APC almost disappeared from the pulp-dentin border after the deposition of reparative dentin on the preoperative dentin in cavity preparation and tooth replantation models. These experimental data imply the putative role of APC in odontoblast differentiation during pulpal regeneration, based on a temporal appearance of the APC along the pulp-dentin border during pulp regeneration after tooth injury.