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Research Article

Possibility of Notch Signaling Role in the Cell Differentiation of Experimentally Induced Periodontal Polyp

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Abstract

In our previous examination, the experimentally induced periodontal polyp in mice was examined the cytological dynamics of the lesion by immunohistochemistry using Green Fluorescence Protein bone marrow-transplanted model mice. Our data indicated that the cells in granulation tissue are mainly from migration of undifferentiated mesenchymal cells of the bone marrow, and differentiate into the tissue-specified cells. Thus, in the present examination using the same methods using ddY mice, we examined the expression of Notch signaling in the fibroblastic cells of the lesions. Histopathological examination revealed the fibroblasts with some round cells and blood vessels were proliferated in the granulation tissue, experimental ranges from at 2 weeks to 6 months. Immunohistochemical staining of Notch1 revealed that the protein was expressed in almost spindle-shaped cells. The result suggests that the periodontal ligament fibroblasts in granulation tissue were expressed Notch1 protein. The fibroblasts in the periodontal polyp granulation tissue were differentiated in the periodontal ligament-specified cells from bone marrow-derived mesenchymal cells. Further examination is needed. However, the data strongly suggests that the cell differentiation within the periodontal polyp was controlled by Notch signaling.

Keywords: Periodontal polyp, Periodontal ligament, Cell differentiation, Notch signaling, Bone marrow-derived cells, Bone marrow-transplanted model, Immunohistochemistry

Introduction

In usual dental clinical practice, perforation of floor of the dental pulp suddenly occurred during a dental treatment. These problems tend to be more in pediatric dentistry. In case of a large perforation is chronic granulomatous growth [1]. Granulation tissue grows in the Periodontal Ligament (PDL) resion from the perforated dentin causing periodontal polyp. Regarding our previous histopathological and immunohistochemical examinations were done [2-5]. The data using an experimental system on GFP mouse bone marrow transplantation model, revealed that the cells were derived from mesenchymal cells of the bone marrow. Furthermore, these cells differentiated into the tissue specific PDL-fiblroblsats and blood capillary endotherial cells, et al.

Notch1 is membrane-bounded protein, which regulates the differentiation gen for changing the cell type [6]. However, there have been no reports on the component-cells of periodontal polyp-granulation tissues [7,8]. In general, Notch signaling is necessary for cell fate determination, cell proliferation and differentiation [9,10].

Therfore in this study, we examined using the method of Matsuda, et al. [7,11]. We examine the expression of Notch1 in the experimentally induced pulp polyp component-cells, using observation of histopathology and immunohistochemistry methods.

Materials and Methods

Animals

A total of 12 ddY mice were from Japan SLC Inc. (Hamamatsu, Japan). Pentobarbital sodium (Somnopentyl, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan) was injected into the abdominal cavity of mouse for general anesthesia.

Perforation of the floor of pulp chamber

The animal was fixed on the hand-made experimental plate and hole was drilled

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on the crown of maxillary left first molar using ½ round bur (Dentsply Sankin Co., Ltd., Tokyo, Japan) to create a perforation of floor of the dental pulp.

The present study was approved by the Animal Experiment Committee of Matsumoto Dental University and Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.

Histopathological preparation

The animals were sacrificed at 2 weeks, 1 month, 3 months and 6 months (Table 1). After each experimental period, the mass was excised, fixed in 4 % neutral buffered formalin solution and then demineralized in 10 % EDTA solution. After washing, tissues were dehydrated in increasing alcohol series, embedded in paraffin and sectioned serially into 4 μm. Then after, sections were deparaffinized and stained with Hematoxylin and Eosin (HE) stain.

Immunohistochemistry

Immunohistochemistry (IHC) was done using Notch1 (Notch1 intercellular domain1: NICD) proteins. Briefly, deparaffinized sections were immersed in xylene, pre-treated in protease solution (Histofine protease, Nichirei Biosciences, Tokyo, Japan) for 5 min at room temperature. This was followed by endogenous peroxidase blocking reagent (peroxidase blocking, Dako Japan Co, Ltd, Tokyo, Japan) for 10 min at room temperature and by non-specific blocking reagent (Protein Blocking, serum-free, Dako Japan Co, Ltd, Tokyo, Japan) for 20 min at room temperature.

Table 1: Experimental Animals and the Periods.

Periods	2 weeks	1 month	3 months	6 months	Total
Animals	3	3	3	3	12

Hereinafter, primary antibody (NICD: Notch1 antibody ab52301 Abcam, Cambridge, UK; 1/100) was used at 4°C for 16 hours. Peroxidase-labeled polymer of primary antibody (Simple Stain Mouse MAX-PO®, Nichirei Biosciences, Co., Tokyo, Japan) was allowed to react for 30 min at room temperature and was developed using DAB (Liquid DAB + Substrate Chromogen System, Dako Japan Co, Ltd, Tokyo, Japan).

Results

Histopathological observation

At 2 weeks, the spindle-shaped cell proliferation was evident with some neutrophils in the specimens. Within these cells, the relatively round nucleus-having cell and some capillaries were observed (Figure 1a-c). Squamous epithelial cells with distinct intercellular bridges which covering the outermost layer of the polyp. At 1 to 6 months-specimens, the granulation tissue directly underneath the perforation became larger and grew inside the pulp chamber; the outermost layer is covered with stratified squamous epithelium. In some cases, neutrophils-rich inflammatory lesion was still evident. The periphery of the areas, the spindle cells with round cells were presented as covering the areas (Figure 1d). At 3 to 6 months, there were observed continuous growth of periodontal polyp, however the collagen bundles were slightly increased, and capillaries slightly decreased.

IHC examination of NICD Protein expression

Regarding the 2 week-specimens, elongated and spindle-shaped cells with spindle nucleus were positive to Notch1. (Figure 1a,b). From 1 to 6 month specimens, many Notch1-positive cells were present in the periodontal polyp underneath the perforation. At 3 months, the spindle-shaped cells were also positive to Notch1. Notch1-positive reaction was continuously

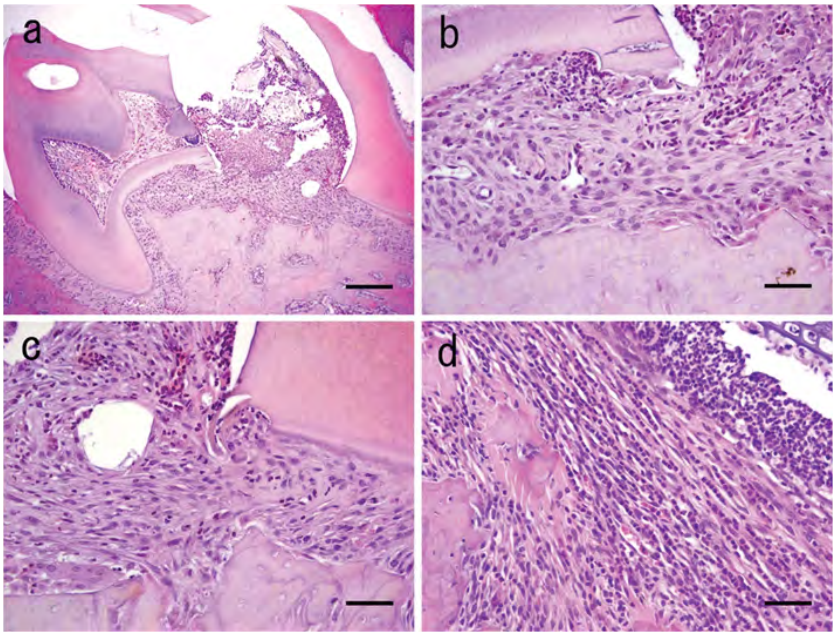

Figure 1: Histopathological features stained by HE.

a: Hole vie of the lesion at 2week-specimen, scale bar=200μm; b: Enlarged view of Figure 1a, scale bar=50μm; c: Enlarged view of Figure 1a, scale bar=50μm; d: Inflammatory lesion and the peripheral tissues of 6 month-specimen, scale bar=50μm.

detected, especially regarding the areas covering the neutrophils rich lesions at 6 months (Figure 1c,d).

In contrast, as observed the control, the dental pulpal tissues of the non-treated teeth was completely negative, although some non-specific positive reactions were existed (Figure 1e,f). Furthermore, the physiological periodontal ligamental tissues slightly positively to Notch1 (Figure 2e).

Discussion

In dental clinical practice, especially in the pediatric dentistry, accidental perforation of floor of the dental pulp is contingent during endodontic treatment. Regarding the lesions, many animal using experimental studies presented the formation of inflammatory lesion as a consequence of perforation of the floor of the pulp chamber. There are some examinations have done, and the published data showed the histopathological analysis and treatment following perforation at the furcation area [4,5]. A detailed histopathological examination showed the continuous growth of granulation tissue in the periodontal ligament [12]. Moreover, to separate and distinguish the periodontal polyp from the growth of the pulp tissue is difficult. Although previous studies have been made, the focus was just on histopathological examination and the origin of the cellular components of the granulation tissue at the furcation was not mentioned.

Previously, we examined using Tsujigiwa-model of GFP bone marrow-transplanted mouse [13], our many published papers suggested that transplanted-bone marrow-derived cells migrated into the periodontal ligament tissues and then differentiated into the tissue specific cells. Muraoka et al. [14] showed that bone marrow-derived cells migrated to the periodontal tissue and then differentiated into periodontal ligament component cells like macrophages and osteoclasts. Moreover, Tomida et al. [15] described the pluripotency of bone marrow-derived cells, which migrated into the periodontal tissue after orthodontic mechanical stress load application. Likewise, Kaneko et al. [16] cited the differentiation of cells into cellular components of the periodontal tissue. Furthermore, Matsuda et al. [1,7] reported that cells having spindle-shaped nucleus, blood vessels and multinucleated giant cells in areas of bone resorption immediately below the perforation were positive to GFP using the model experiments. Moreover, GFP-positive cells were also spotted to have infiltrated the epithelium, although there were no positive cells in the

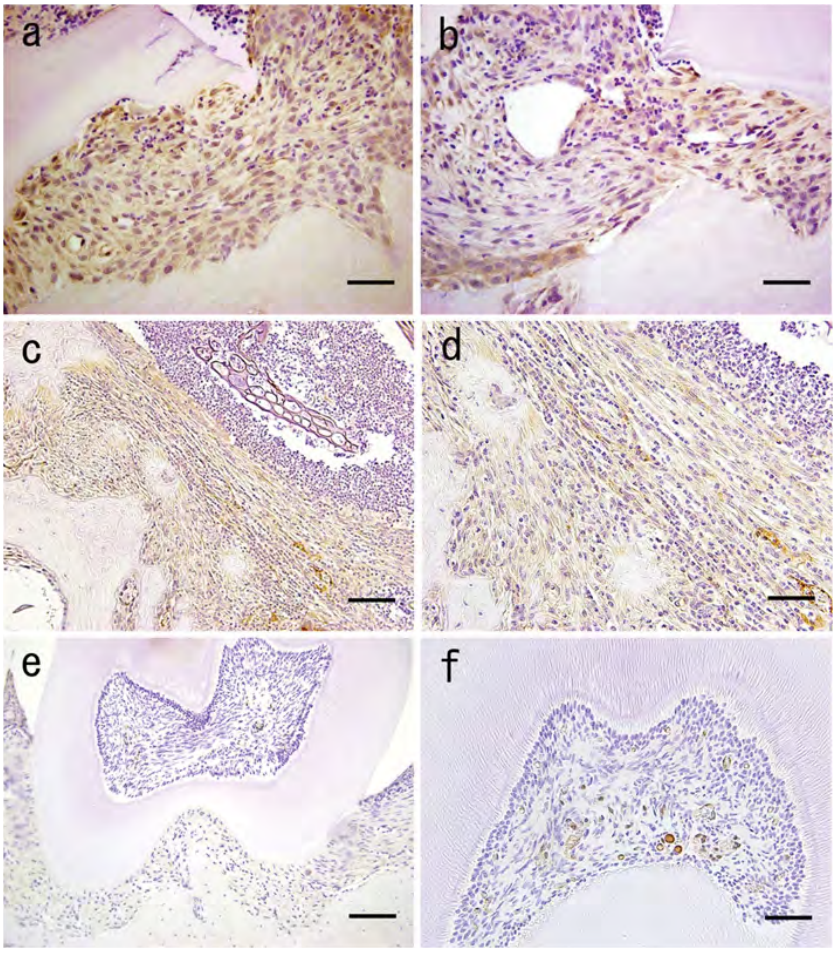

Figure 2: IHC images of Notch expression.

a: The almost same area of Figure 1b, scale bar=50μm; b: The almost same area of Figure 1c, scale bar=50μm; c: Same area of Figure 1d, scale bar=100μm; d: Enlarged view of Figure 1c, scale bar=50μm; e: Control areas (dental pulp and periodontal ligament tissues) of 6 month-specimen, scale bar=100μm; f: Control areas (dental pulp and periodontal ligament tissues) of 6 month-specimen, scale bar=50μm.

epithelium. Therefore, the cells are considered to be dendritic cells. Thus, it would also be reasonable to consider that the cells were derived from mesenchymal cells of the bone marrow. In order to identify the GFP-positive cells, double FIHC staining for each marker was performed.

The examination results showed that the histopathological features were also nearly the same as Matsuda et al. [7]. That is inflammatory cells such as neutrophils were present in response to the physiological mechanism of the periodontal tissue. This was followed by the increase in the fiber component of the granulation tissue as the number of inflammatory cells decreased. Experimental perforation of floor of the dental pulp chambers in mice caused a slight initial superlative inflammation leading to granulation tissue growth, which mainly component are fibroblasts, collagen bundles and capillaries, with slightly a little chronic inflammation [7].

In the also same examination [7], we reported that some of the capillaries were obtained from the migration and differentiation of undifferentiated mesenchymal cells from the bone marrow cells. From the above results and overall consideration, fibroblasts, periodontal ligament fibroblasts and blood vessels are essential in the growth of periodontal polyp due to perforation on the floor of the pulp chamber. Furthermore, the osteoclasts observed on the surface of the alveolar bone beneath the perforation originated from transplanted bone marrow cells. These conclusion lead to us to examine the relationship between the Notch signaling and the cell differentiation of the lesions.

Generally Notch1 is an important regulation signaling of morphogenesis. It was reported Notch1 is a transmembrane protein necessary for cell fate determination, etc. [6]. Disruption of Notch1 ligands and recepters as well as downstream signaling components of Notch1 pathway have been implicated in a lot of developmental defects and pathological conditions [17,18]. Notch functions in all progenitor cells give rise to the many cells and tissues [19,20]. Futhermore, the Notch family is slightly-conserved family of cell surface signaling molecules [21]. Thus, we examined the relationship between the cell differentiation in the periodontal polyp component cells and Notch-signaling in the present study.

However, IHC examinations maybe need the statistical analysis. The statistical analysis maybe deal the more detail results from these examination. Therefore, if possible in future examinations, we got the results, we present as the other manuscript. Furthermore, the downstream of the Notch signaling pathways are also interesting phenomenon. Therefore, we will examine the phenomenon in the next stage of the examination.

In this examination, above mentioned discussion, the precaution cells of the PDL fibroblasts were coming from the bone marrow tissues, and then differentiate into the periodontal ligament fibroblasts. This phenomenon was thought in our previous examinations [7,11]. According to the present results, (1) Spindle-shaped-fibroblastic cell of the polyp polyp tissues were almost Notch1 positively reacted. (2) These reactions strongly suggested the cell differentiation was caused by the Notch1-signaling. The dental pulp tissues is completely negative, which originated from neural crest cell-derived odontogenic tissues as same as the periodontal ligament tissues. The fact is well known. However, there were completely negative reactions in the pulp tissues as the control specimens. This was very important features. (3) Regarding the physiological periodontal ligament tissues weakly reacted to the Notch1. The reactions means that the periodontal ligament tissues are always received and remodeling the tissues caused by the forces of the occlusal forces, etc.

Further examination is needed to the mechanisms of the role of Notch signaling. In the near future date, the results will be presented.

Conclusion

In the examination, we observed the Notch1-protein expression within the cells of experimentally induced pulp polyp using IHC. The IHC results show that the Notch1-Protein expressed within the fibroblastic cells of the experimentally induced periodontal polyp tissues. Thus, the data strongly suggested that cell differentiation was caused by Notch-signaling.

Acknowledgments

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Conflict of Interest

The authors have declared that no COI existed.

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