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Original Article

Histopathological analysis of the differential diagnosis of peripheral odontogenic fibroma from fibrous epulis

Kei Manabe [a, 1], Mayumi Yakeishi [b, 1], Wakako Sakaguchi [a], Juri Saruta [a, *], Keiichi Tsukinoki [a]

[a] Division of Environmental Pathology, Department of Oral Science, Graduate School of Dentistry, Kanagawa Dental University, 82 Inaoka-cho, Yokosuka, Kanagawa, 238-8580, Japan
[b] Department of Pathology, Yokosuka Kyosai Hospital, 1-16 Yonegahamadori, Yokosuka, Kanagawa, 238-8558, Japan

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ABSTRACT

Objectives: Peripheral odontogenic fibroma (POF) is a relatively rare odontogenic tumor of the gingiva. Although its histological differential diagnosis from fibrous epulis (FE) is important, no study has reported the differences in their expression of immunohistochemical markers. Here, we compared the expression of tumor markers that are frequently used for the differential diagnosis of fibroproliferative lesions between POF and FE.

Methods: Forty cases were selected, including 20 POF and 20 FE cases. CD34, B cell lymphoma (Bcl)-2, and Ki-67 were used as markers for immunohistochemical examination. The positive cell ratio was calculated, and Mann-Whitney *U* test was performed for statistical analysis.

Results: POF and FE were negative for CD34 expression but showed Bcl-2 and Ki-67 expression. The ratio of Bcl-2- and Ki-67-positive cells was significantly higher in POF than in FE ($p < 0.001$).

Conclusions: POF is CD34 negative, and Bcl-2 and Ki-67 positive-cell ratio differs between POF and FE, suggesting that these proteins may serve as immunohistochemical markers for the differential diagnosis of POF.

1. Introduction

Peripheral odontogenic fibroma (POF) is defined as an odontogenic mesenchymal tumor of the gingiva. It is the counterpart of central odontogenic fibroma [1]. Histopathological features of POF include proliferation of fibroblasts and presence of collagenous stroma-containing inactive odontogenic epithelium. Although POF is relatively rare, 422 cases of POF have been reported by Heithersay GS et al. [2,3]. However, POF incidence may be more than that reported, owing to the difficulty in differentiation between true fibromas and hyperplastic lesions [4]. The recurrence rate of POF after surgery varies from 29 out of 58 (50%), as documented by Ritwik et al. [5], to 1 out of 30 (3%), as determined by De Villiers Slabbert H and Altini M [6]. The recurred cases often require long follow-ups. Thus, POF requires detailed differential diagnosis, although no reports have ever documented the immunohistochemical characteristics of POF.

In contrast, solitary fibrous tumor (SFT) is the most common type of fibrous tumor that is considered to be intermediately malignant in the relatively wide spectrum of benign to malignant forms [7]. Hence, SFT should be differentiated from various spindle cell tumors [8]. Using immunostaining, CD34 and B cell lymphoma (Bcl)-2 have been identified as the most reliable markers [8]. In particular, CD34 that is expressed on undifferentiated mesenchymal cells and fibroblasts has been recognized in many fibroblastic tumors and is a representative marker for the differential diagnosis of fibrous tumors [9,10]. As proliferative activity is associated with disease recurrence, the proliferation marker Ki-67 is widely employed for pathological diagnosis, including for the determination of malignancy [11].

Here, we investigated the immunohistochemical expression of CD34, Bcl-2, and the proliferation marker Ki-67 in POF to clarify their significance in differential diagnosis. POF is often clinically diagnosed as epulis [12,13]; therefore, the most commonly

Abbreviations: POF, peripheral odontogenic fibroma; FE, fibrous epulis; Bcl-2, B cell lymphoma-2; SFT, solitary fibrous tumor.

* Corresponding author.
E-mail address: saruta@kdu.ac.jp (J. Saruta).

[1] Kei Manabe and Mayumi Yakeishi contributed equally as first authors.

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considered disorder in the differential diagnosis of POF, fibrous epulis (FE), was designated as control.

2. Materials and methods

2.1. Case selection

Twenty samples of POFs were obtained from various cases of fibroproliferative lesions collected at the Kanagawa Dental University Yokohama Clinic and Department of Pathology. In addition, 20 FE samples obtained from Yokohama clinic were used as controls. The definitive diagnosis of POF was based on the guidelines set forth by the World Health Organization (WHO) classification [1]. We confirmed the presence of keratin expression and, hence, odontogenic epithelial islands in these cases.

2.2. Immunohistochemical staining

Samples were fixed with 10% neutral buffer formalin solution. Immunohistochemical analysis was performed on a series of sections obtained from the tissues embedded in paraffin blocks. The expression of CD34, Bcl-2, and Ki-67 was determined using mouse anti-human CD34 monoclonal antibody (clone Q-BEND10, dilution 1:200, Immunotech, Hialeah, FL, USA), mouse anti-human Bcl-2 monoclonal antibody (clone 124, dilution 1:50, DAKO Cytomation, Glostrup, Denmark), and mouse anti-human Ki-67 monoclonal antibody (clone MIB-1, dilution 1:100, MBL Co., LTD, Nagoya, Japan), respectively, as primary antibodies. The antigen retrieval of Bcl-2 and Ki-67 was performed by incubating the sections immersed in 0.01 M citric acid buffer solution (pH 6.0) in an autoclave at 120 °C for 5 min. After incubation with secondary antibodies, staining was performed as per the manual of Histofine simple stain MAX-PO (M) (Nichirei, Tokyo, Japan).

2.3. Cell measurement method

A total of 1000 cells were counted under a 40 × objective lens of a microscope (Olympus B×41, Olympus, Tokyo, Japan) from one edge of the lesion to the other edge (including the center) to calculate the number of positive cells, as previously described [14]. While determining the number of CD34-positive cells, vascular endothelial cells in the section were selected as internal controls, and the cells with intensity similar to that of vascular endothelial cells were deemed positive. Lymphocytes and squamous basal cells in the section were considered as negative control in the case of Bcl-2 and Ki-67, respectively. The cells showing the same color intensity were deemed positive for biomarker expression.

2.4. Statistical analysis

Statistical analyses were carried out using SPSS (Version 22.0; SPSS Inc., Chicago, IL, USA) statistics program. The ratio of Bcl-2- and Ki-67-positive cells was analyzed using the Mann-Whitney *U*-tests. A value of p less than or equal to 0.05 was considered statistically significant.

3. Results

3.1. Histological findings of POF and FE

POF was observed in the subepithelial layer without any fibrous capsules (Fig. 1A). Subepithelial lamina propria showed a dense collagenous tissue that was clearly demarcated from the tumor (Fig. 1A). The tumor mass comprised spindle-shaped cells characterized with mild atypia and scanty collagen fibers (Fig. 1B). Odontogenic epithelial islands surrounded by hyaline degeneration were evident in the tumor mass (Fig. 1B). FE showed proliferation of fibrous tissue with mild chronic inflammatory cell infiltration (data not shown).

3.2. CD34 expression

The vascular endothelial cells showed positive expression of CD34 (Fig. 2A–C). Fibroblasts of the lamina propria showed no immunohistochemical reaction (Fig. 2A). In addition, the spindle-shaped cells of POF (Fig. 2B) and FE (Fig. 2C) showed no expression of CD34.

3.3. Bcl-2 expression

Clear Bcl-2-positive staining was observed in the cytoplasm of lymphocytes (Fig. 3A–C). Fibroblasts of lamina propria showed no positive immunohistochemical staining (Fig. 3A). In POF (Fig. 3B), Bcl-2 expression was diffused in spindle-shaped cells. In contrast, Bcl-2 expression in spindle-shaped cells of FE was weaker than that observed in lymphocytes (arrow) (Fig. 3C). The Bcl-2-positive cell ratio was significantly higher in POF than in FE ($p < 0.001$) (Fig. 4). The median of Bcl-2-positive cell ratio was 86.25% in POF; the maximum and minimum values were 98.3% and 63.1%, respectively. Bcl-2 expression was detected in all 20 POF cases. The median of Bcl-2-positive cell ratio was 3.10% in FE, with the maximum and minimum values of 14.6% and 0%, respectively. In addition, Bcl-2 expression was detected in 17 of 20 FE cases. Bcl-2 was weakly expressed by the odontogenic epithelium of POF.

3.4. Ki-67 expression

Clear positive Ki-67 expression was observed in the nuclei of cells from the basal and parabasal layers in the covered squamous epithelium (Fig. 5A). Ki-67 expression was also detected in the nuclei of spindle-shaped cells of POF (Fig. 5B). In addition, positive expression was noted in the nuclei of spindle-shaped cells from fibrous tissues rich in collagen fibers of FE (Fig. 5C). The Ki-67-positive cell ratio was significantly higher in POF than in FE ($p < 0.001$) (Fig. 6). The median of Ki-67-positive cell ratio was 0.40% in POF and the maximum and minimum values were 2.70% and 0.10%, respectively. Ki-67 expression was detected in all 20 cases of POF. The median of Ki-67-positive cell ratio was 0.015% in FE and the maximum and minimum values were 0.70% and 0%, respectively. Ki-67 was expressed in 19 of 20 FE cases.

4. Discussion

The CD34 antigen is a single-chain transmembrane glycoprotein with a molecular size of about 110 kDa. It has two structurally different domains on cell membranes [10]. CD34 is expressed in SFT [7,8], elastofibroma [15], irritation fibroma [9], sclerotic fibroma [16], and dermatofibrosarcoma protuberans [17] and commonly used as an important marker for the differential diagnosis of fibrous tumors. However, the lack of expression of CD34 in the tumor cells of collagenous fibroma [18] has prompted the existence of CD34-negative fibrous tumors. In the present study, CD34 was undetected in POF and FE, which originate from the fibroblasts in the lamina propria of gingival mucosa or periodontal ligament. POF and FE are thought to have the same origin, which may explain the observation related to CD34 expression. The fibroblasts in the

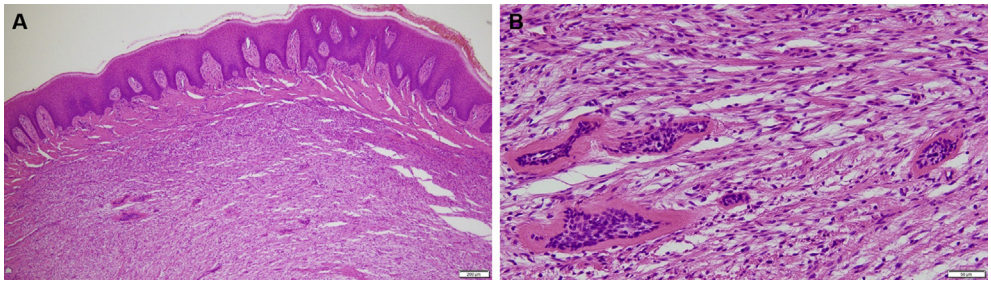

Fig. 1. Hematoxylin and eosin-stained images showing histological findings of peripheral odontogenic fibroma (POF). (A) Low-power view reveals the tumor mass beneath the gingival epithelium (scale bar = 200 μm). (B) Odontogenic epithelial islands scattered throughout the tumor (scale bar = 50 μm).

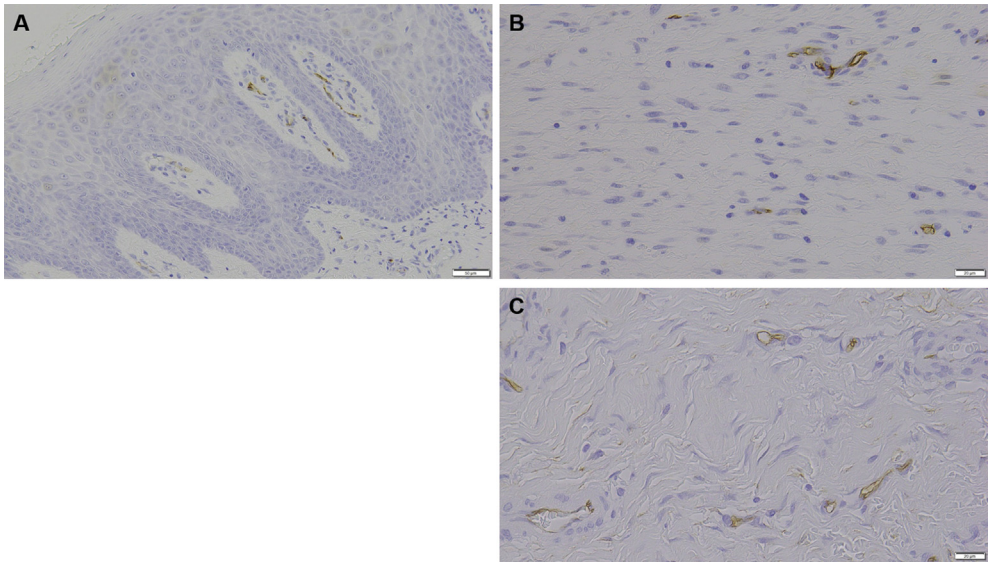

Fig. 2. CD34 expression. Endothelial cells serving as internal controls are positive for CD34 expression (A–C). (A) Fibroblasts in the lamina propria of gingiva are negative for CD34 expression (scale bar = 50 μm). (B) Spindle-shaped tumor cells in peripheral odontogenic fibroma show no expression of CD34 (scale bar = 20 μm). (C) Spindle-shaped fibrous cells in fibrous epulis are negative for CD34 (scale bar = 20 μm).

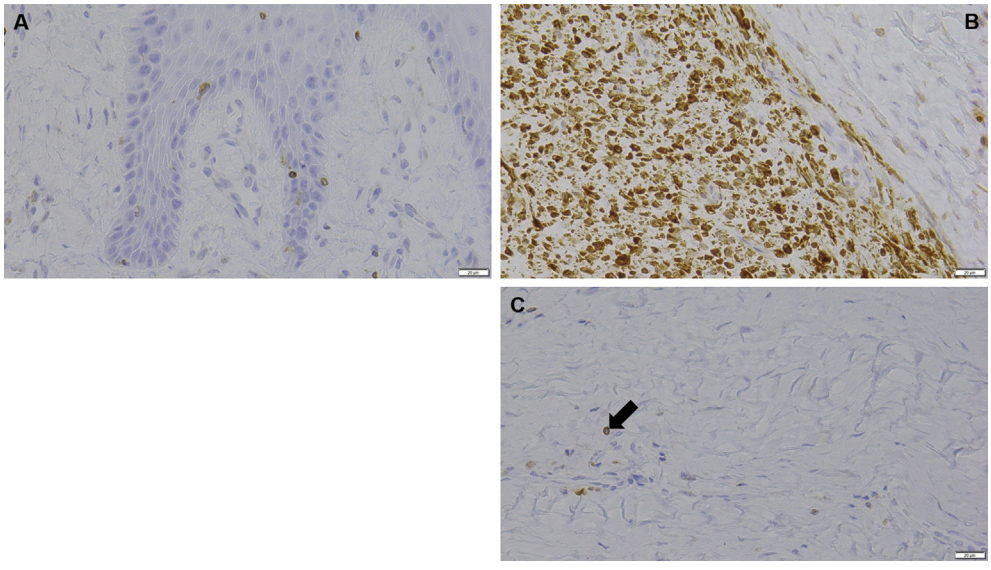

Fig. 3. Bcl-2 expression. Lymphocytes serving as internal controls are positive for Bcl-2 expression (A and C). (A) Fibroblasts in the lamina propria of gingiva show no expression of Bcl-2 (scale bar = 20 μm). (B) Tumor cells in the peripheral ossifying fibroma are strongly positive for Bcl-2 (scale bar = 20 μm). Fibrous cells in lamina propria are Bcl-2 negative. (C) Spindle-shaped cells in fibrous epulis show weakly positive or negative Bcl-2 expression (scale bar = 20 μm). Arrow shows lymphocyte.

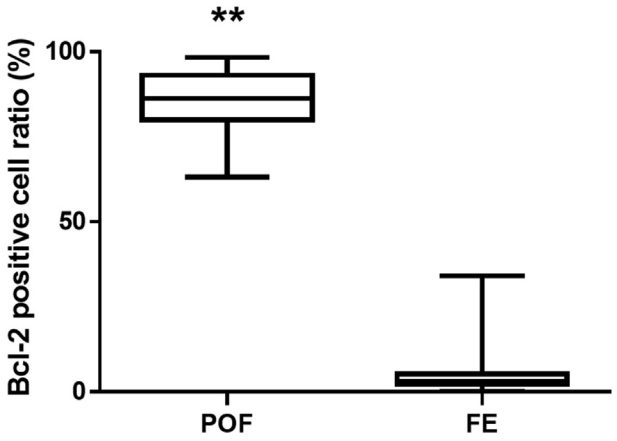

Fig. 4. Bcl-2-positive cell ratio. Graph shows the significant difference in Bcl-2-positive cell ratio between peripheral odontogenic fibroma (POF) (n = 20) and fibrous epulis (FE) (n = 20). Box plots represent the smallest observation; lower, median (horizontal bar), and upper quartile; and largest observation. (**$p < 0.001$, Mann-Whitney *U* test).

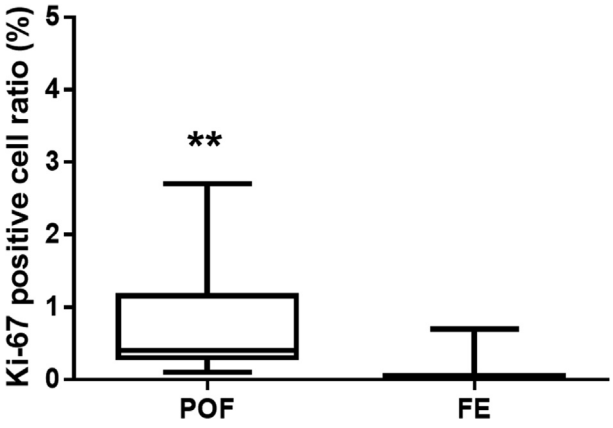

Fig. 6. Ki-67-positive cell ratio. Graph reveals the significant difference in Ki-67-positive cell ratio between peripheral odontogenic fibroma (POF) (n = 20) and fibrous epulis (FE) (n = 20). Box plots represent the smallest observation; lower, median (horizontal bar), and upper quartile; and largest observation (**$p < 0.001$, Mann-Whitney *U* test).

lamina propria of gingival mucosa were also negative for CD34 expression. Irritation fibromas of the palate that do not exhibit the same submucosal tissue as the gingiva have also been reported to be negative for CD34 [9]. Furthermore, many reports suggest that stem cells of the periodontal ligament lack CD34 expression [19]. Therefore, it is possible that POF and FE are diseases arising from the proliferation of CD34-negative fibroblasts.

Bcl-2 is an oncogene derived from gene translocation and is involved in the development of follicular B lymphoma [20]. Its function is to prolong cell survival through the inhibition of apoptosis [20]. Bcl-2 is reported to be involved in the suppression of apoptosis in benign oral tumors [21]. Although EF showed very few cells positive for Bcl-2 expression at the lymphocyte level, strong expression of Bcl-2 was detected in POF and the difference was statistically significant. Suzuki et al. reported that the cells of dental papilla or tooth follicles, which are the source of origin of POF, lack Bcl-2 expression [22]. Thus, Bcl-2-positive cells of POF may develop as a result of autonomous growth, suggestive of their difference from hyperplastic lesions such as FE.

Ki-67 is a cell proliferation marker widely used in the pathological diagnosis of diseases with different degrees of cell proliferation [14]. Ki-67 expression correlates with the degree of differentiation, vascular invasion, lymph node metastasis, and recurrence in many tumors [23]. Here we found that POF showed a significantly higher ratio of positive cells as compared with FE. The proportion of Ki-67-positive cells was shown to be less than 5% in ameloblastoma with increased chances of recurrence [24]. POF showed low proliferative activity, as the proportion of Ki-67 cells was equal to less than 1%. However, as the proliferative activity of POF was higher than that of FE, our study result reveals the importance of long-term follow-up in patients with POF considering the increased risk of recurrence in such cases.

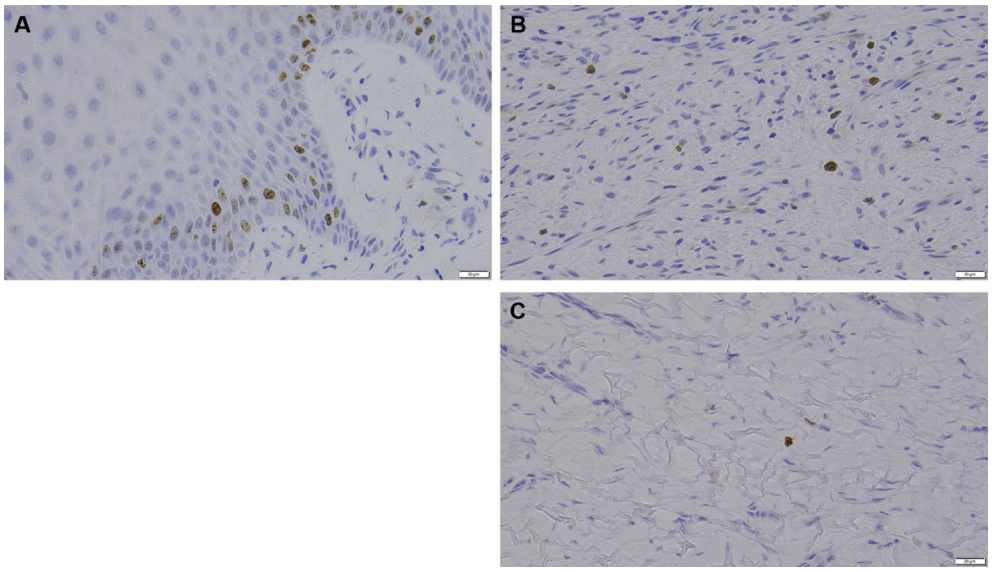

Fig. 5. Ki-67 expression. (A) Cell nuclei in the basal cell layer of covered stratified squamous epithelium show positive Ki-67 expression (scale bar = 20 μm). (B) Nuclei of the tumor cells in peripheral odontogenic fibroma are positive for Ki-67 expression (scale bar = 20 μm). (C) Nuclei of the spindle-shaped cells of fibrous epulis show weak staining for Ki-67 (scale bar = 20 μm).

5. Conclusion

In summary, POFs were negative for CD34 expression and showed a high ratio of Bcl-2-positive cells. The biomarker expression profile of POFs was different from that of FE. These markers could be useful as immunohistochemical molecules for the differential diagnosis of POF and other fibroproliferative diseases such as FE and SFT. POF exhibited higher proliferative activity than FE, indicating the importance of long-term follow-up to prevent recurrence in such cases.

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Ethical approval

This research has received approval from the Kanagawa Dental University Research Ethics Review Board (No. 340 and 596).

Conflicts of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Kei Manabe: Data curation, Formal analysis, Investigation, Writing - original draft. **Mayumi Yakeishi:** Data curation, Investigation, Methodology, Writing - original draft. **Wakako Sakaguchi:** Investigation. **Juri Saruta:** Resources, Supervision, Validation, Writing - review & editing. **Keiichi Tsukinoki:** Conceptualization, Validation, Project administration, Writing - review & editing.

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