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Original Article

Comparison of CD34 expression in fibrous reactive hyperplasia and healthy oral mucosa

Masako Nagasaki [a, 1], Wakako Sakaguchi [a, 1], Shinya Fuchida [b], Nobuhisa Kubota [a], Juri Saruta [a], Kenji Suzuki [c], Hiroshi Iwabuchi [c], Masaru Kobayashi [c], Shigeru Ishii [d], Atsushi Nakamura [d], Yuko Yamamoto [e], Keiichi Tsukinoki [a, *]

[a] Division of Environmental Pathology, Department of Oral Science, Japan
[b] Division of Dental Sociology, Department of Disaster Medicine, Dental Sociology, Japan
[c] Division of Oral and Maxillofacial Surgery, Department of Dentomaxillofacial Diagnosis, Treatment Graduate School of Dentistry, Kanagawa Dental University, 82 Inaoka-cho, Yokosuka, Kanagawa, 238-8580, Japan
[d] Department of Oral Surgery, Kanagawa Dental University Yokohama Clinic, 3-31-6 Tsuruya-cho, Yokohama, Kanagawa, 221-0835, Japan
[e] Division of Dental Hygiene, Kanagawa Dental University Junior College, 82 Inaoka-cho, Yokosuka, Kanagawa, 238-8580, Japan

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ABSTRACT

Objectives: Fibrous reactive hyperplasia (FRH) is a common fibrous lesion in the oral cavity. The disease characteristics of FRH, including the expression patterns of CD34, which is a well-known fibroblast marker, have not been investigated in detail. Therefore, in this study, we aimed to investigate the characteristics of FRH compared to those of the healthy mucosa, based on CD34 expression profiles.
Methods: CD34 expression was analyzed at the protein and mRNA levels using immunohistochemistry, quantitative polymerase chain reaction, and in situ hybridization (ISH).
Results: CD34 was not expressed in the lamina propria of the oral mucosa, but was commonly observed in submucosal fibroblasts. CD34-positive fibroblasts were commonly observed in FRH. A total of 17 out of 19 cases (89.5%) were CD34-positive. Furthermore, we identified a significant difference in the ratio of CD34-positive cells between the healthy and FRH tissues. Quantitative polymerase chain reaction showed that *CD34* mRNA was expressed in all cases of FRH, and *CD34* mRNA expression in FRH samples was found to be localized to spindle-shaped fibroblasts, as determined by ISH. A positive correlation was also found between the *CD34* mRNA levels and the proportion of the CD34-positive cells.
Conclusions: These findings suggest that the increase in collagen synthesis in CD34-positive fibroblasts in the submucosa leads to the development of FRH. To our knowledge, this is the first report confirming the mRNA expression patterns of *CD34* in FRH.

1. Introduction

Fibroproliferative lesions originating from fibroblasts are common in the oral cavity [1]. There are two types of fibroproliferative lesions: true fibromas and fibrous reactive hyperplasia (FRH) [2]. True tumors include solitary fibrous tumors (SFT) [3], collagenous fibroma (CF) [4], sclerotic fibroma [5], and elastofibroma (EF) [6]. These lesions are known to have a mesenchymal origin with no neural or endothelial association [3]. The major types of FRH in the oral cavity include irradiation, traumatic, and denture fibroma, and occur more frequently than true fibromas [2]. FRH are characterized by increase of fibrous connective tissue containing collagen matrix and fibroblast without atypia. Hyperplasia of fibrous connective tissue occurrences various mechanical irritations. Histologically, FRH is mainly composed of dense collagen fibers and displays various degrees of spindle-shaped fibroblast and fibrocyte [2]. Furthermore, despite the high frequency of lesions resulting from FRH, its characteristics have not yet been examined in detail.

Recently, CD34 was established as an antigen associated with fibroblasts [7]. CD34 expression analysis is the most common method used for the examination of fibroproliferative lesions because it is often applied for the differential diagnosis of

* Corresponding author. Department of Oral Science, Graduate School of Dentistry, Kanagawa Dental University, 82, Inaoka-cho, Yokosuka, Kanagawa, 238-8580, Japan. Fax: +81 46 822 8855.
E-mail address: tsukinoki@kdu.ac.jp (K. Tsukinoki).
[1] Masako Nagasaki and Wakako Sakaguchi contributed equally to this study.

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fibroproliferative lesions, including SFT [8] and dermatofibrosarcoma protuberans [9]. Although CD34 is an important molecule involved in fibroblast differentiation, there are also some CD34-negative fibroproliferative lesions, including CF [10], peripheral odontogenic fibroma, and fibrous epulides [11]. The role of CD34 expression in fibroproliferative lesions remains unclear, and minimal research has been performed on CD34 expression patterns in FRH.

The authors have previously reported that fibroproliferative lesions occurring in the gingiva are CD34-negative [11]. This is thought to be related to the fact that the lamina propria fibroblasts are CD34-negative. On the other hand, the authors have discovered that CD34-positive cells are found in the submucosa, and hypothesized that fibroproliferative lesions that occur on the tongue and buccal mucosa are derived from CD34-positive fibroblasts. Therefore, the purpose of this study was to identify the origin of FRH in comparison with that of healthy submocosa, based on the CD34 expression profile by analyzing mRNA and protein levels.

2. Materials and methods

2.1. Study cohort

A total of 21 resected FRH samples (men: 10, women: 11, mean: 57.3, range: 28–71, Japanese) were selected based on the clinical diagnosis of fibromas at the Kanagawa Dental University Hospital (Kanagawa, Japan), Kanagawa Dental University Yokohama Clinic (Kanagawa, Japan), and Sashiogi Hospital (Saitama, Japan). Samples from the lesions were obtained between 22 May 2018 and 26 May 2019 from patients who provided informed signed consent. In addition, this study was approved (No. 340) by the Kanagawa Dental University Research Ethics Review Board. The pathological diagnostic criteria for FRH defined as non-demarcation of the lesion from the surrounding healthy tissue, increased collagen fibers compared to levels of other cellular components, and the absence of the fibrous septum [12]. The arrangement of thick collagen fibers was defined as irregular, where no multinucleated cells and almost no inflammatory cell infiltration was observed [12]. Based on these criteria, 19 of the 21 cases were diagnosed as FRH, and two cases were diagnosed as collagenous fibromas. Of these 19 cases, seven were located in the tongue, nine in the buccal mucosa, two in the palate, and one in the upper lip. Gingival lesions were not included in this study because fibrous epulides were excluded. Healthy tissues at the resected margins of the collected lesions were obtained for use as healthy mucosal tissue.

2.2. Histological examination and immunohistochemistry

Samples were fixed with 10% neutral buffered formalin, and 5 μm serial sections were sliced from paraffin blocks for pathological diagnosis. Hematoxylin and eosin (H&E) staining and immunohistochemistry was performed to detect CD34 expression. The localization of CD34 was determined using a mouse anti-human CD34 monoclonal primary antibody (Q-BEND10, 1:200; Beckman Coulter, Brea, CA, USA). Endogenous peroxidase removal was performed by treatment with a 3% hydrogen peroxide (H_2O_2) solution for 10 min. Primary antibodies were reacted at room temperature of 22 °C for 1 h. After incubation with the secondary antibody, staining was performed using the Histofine Simple Stain MAX PO (M) Kit (Nichirei, Tokyo, Japan) according to the manufacturer's instructions. Thereafter, samples were incubated with the secondary antibody at temperature of 22 °C for 30 min and visualized with 3,3′-diaminobenzidine and H_2O_2. Finally, the sections were mounted and visualized under a microscope (BX53, Olympus, Tokyo, Japan). Negative control specimens were stained under the same conditions after reacting with normal mouse immunoglobulin (IgG).

2.3. Quantitative analysis of CD34-positive cells

To measure the expression of CD34, vascular endothelial cells were used as an internal standard; cells showing the same staining intensity as that of vascular endothelial cells were considered to be CD34-positive. The submucosa included the reticular layer of the mucosal lamina propria because it is difficult to distinguish the reticular layer of the lamina propria from submucosal connective tissue. In addition, the lamina propria was measured on the papillary layer. Cells were counted after photographing specimens under a light microscope using a 40 × objective lens (Olympus, Tokyo, Japan); CD34-positive and -negative cells were counted independently by two researchers (M. N. and K. T.). Cells were counted in three or more randomly selected quadrants in each image, and more than 300 cells were contained in each quadrant. The positive cell ratio was calculated as the number of positive cells to the total number of cells.

2.4. Quantitative polymerase chain reaction (PCR)

A portion of the collected samples were immediately frozen and stored until required for the next set of experiments. Total RNA was isolated using ISOGEN reagent (Nippon Gene Co., Ltd., Toyama, Japan) according to the manufacturer's instructions. RNA concentrations were then determined using a BioSpec-Nano spectrophotometer (Shimadzu Access Corp, Kanagawa, Japan). cDNA was synthesized from total RNA using a first-strand cDNA synthesis kit (Roche Diagnostics, Basel, Switzerland), and quantitative PCR was performed using a LightCycler 480 System (Roche Diagnostics). The following primers were used to amplify the *CD34* gene sequence: 5′-GTCACCTGGAAATGTTTCAGAC-3′ (forward) and 5′-CTGAGTCAATTTCACTTCTCTGATG-3′ (reverse); primers were designed by the Nippon Gene Research Institute (Sendai, Japan). Quantitative PCR was performed under the following conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 58 °C for 20 s, and 72 °C for 10 s. *β-actin* was used as an internal control using the LightCycler Primer set (Search-LC, Heidelberg, Germany) for normalization (95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s). Gene expression was calculated as the ratio of the expression of *CD34* to the expression of *β-actin*.

2.5. In situ hybridization

In situ hybridization (ISH) was performed using the QuantiGene ViewRNA ISH Cell Assay Kit (Panomics, Inc., Fremont, CA, USA) with paraffin block samples [13,14]. Paraffin sections were baked at 60 °C for 60 min and deparaffinated using xylene. Samples were boiled at 95 °C for 10 min in pre-treatment solution (Affymetrix, Santa Clara, CA, USA) and digested at 40 °C for 20 min with proteinase (1:100; Panomics). Sections were incubated with custom QuantiGene ViewRNA probes for 2 h at 40 °C. Thereafter, the signal was amplified with Pre-Amp and Amp solutions (1:100) and incubated with alkaline phosphatase-conjugated label probe 6 (LP6- AP) (1:1000; Panomics) at 40 °C for 15 min. Samples were then stained with Fast Blue substrate (Panomics) at 40 °C for 30 min. Next, sections were incubated with LP1- AP (1:1000; Panomics) at 40 °C for 15 min and stained with Fast Red substrate (Panomics) at 40 °C for 30 min. Finally, sections were treated with 10% formaldehyde for 5 min at room temperature and rinsed with phosphate-buffered saline (PBS).

2.6. Statistical analysis

The normal distribution of the positive cell ratios was confirmed by the Kolmogorov–Smirnov test, and the average values were compared using the Student's paired *t*-test. The relationship between the FRH-positive cell ratio and the CD34-positive/actin-positive ratio was analyzed using Spearman's rank correlation coefficient because the CD34-positive/actin-positive ratio was not normally distributed by the Kolmogorov–Smirnov test. IBM-SPSS Statistics v23.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. CD34 expression in healthy tissues at the surgical margin

Small capillaries and fibroblasts were observed in the lamina propria of the palate (Fig. 1A). CD34 expression was positive in vascular endothelial cells, but negative in fibroblasts of the lamina propria (Fig. 1B). Similar observations were made in the tongue, buccal lip mucosa, and upper lip mucosa (data not shown).

The submucosa of the tongue contained large blood vessels, fibroblasts, and loose collagen fibers (Fig. 1C). CD34 expression was positive in vascular endothelial cells and fibroblasts (Fig. 1D). Similar observations were made in the buccal mucosa and upper lip (data not shown). No CD34 signals were observed in sections from the negative control samples (data not shown).

3.2. CD34 expression in FRH

Of the 19 cases of FRH, 17 cases were CD34-positive (89.5%), and two cases in the palate were CD34-negative. FRH in the buccal mucosa showed a random distribution of thick collagen fiber bundles, as well as an increase in the production of extracellular matrix components (Fig. 2A). Spindle-shaped fibroblasts were observed along with randomly distributed collagen fibers. Furthermore, CD34-positive expression was observed in the cell membranes and cytoplasm of vascular endothelial cells and fibroblasts (Fig. 2B). Similar observations were made in the tongue and upper lip (data not shown). With regards to FRH in the palate, fibroblasts did not show CD34 expression (data not shown). No CD34 signals were observed in the sections from negative control samples (data not shown).

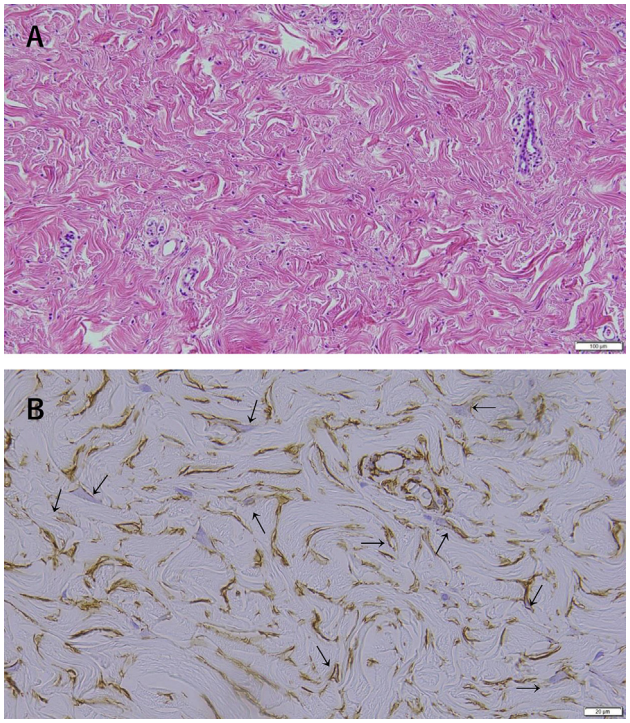

Fig. 2. Representative images of fibrous reactive hyperplasia (FRH) in the buccal mucosa (A) Hematoxylin and eosin (H&E) staining showing dense collagen fibers and fibroblasts in the buccal mucosa (B) Spidle-shaped fibroblasts with CD34 expression in the cell membrane and cytoplasm (arrow). Scale bar = 100 μm (A); Scale bar = 20 μm (B).

3.3. CD34-positive cell ratio

The CD34-positive cell ratio was 63.21 ± 14.97% in healthy tissues at the surgical margins. In contrast, the CD34-positive ratio of

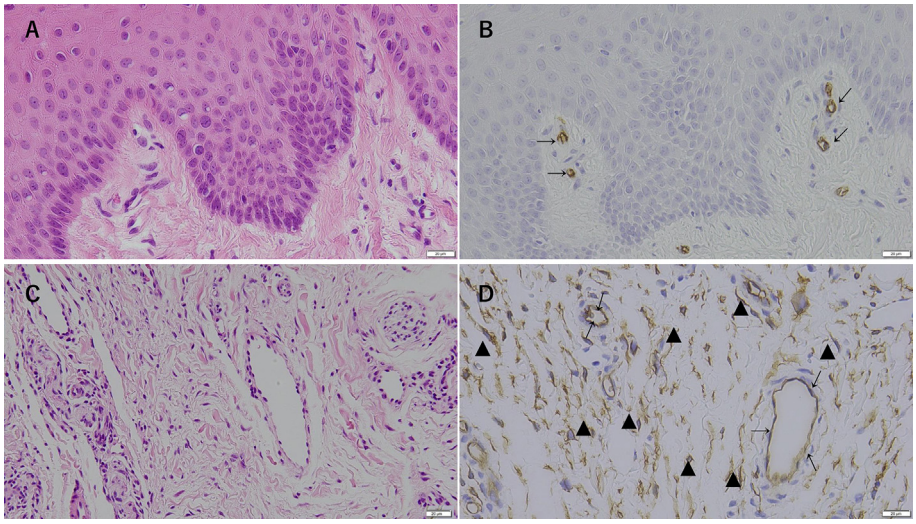

Fig. 1. Representative images of high-magnification samples of healthy tissue (A) Hematoxylin and eosin (H&E) staining showing the lamina propria of palate mucosa. This case does not include submucosa (B) No CD34 expression is observed in fibroblasts derived from the lamina propria of the palate; however, CD34 expression is observed in vascular endothelial cells (arrow) (C) H&E staining showing loose connective tissue, including capillary and fibroblast tissues (D) Immunohistochemical staining of CD34 in vascular endothelial cells (arrow) and fibroblasts (arrowhead). Scale bar = 20 μm.

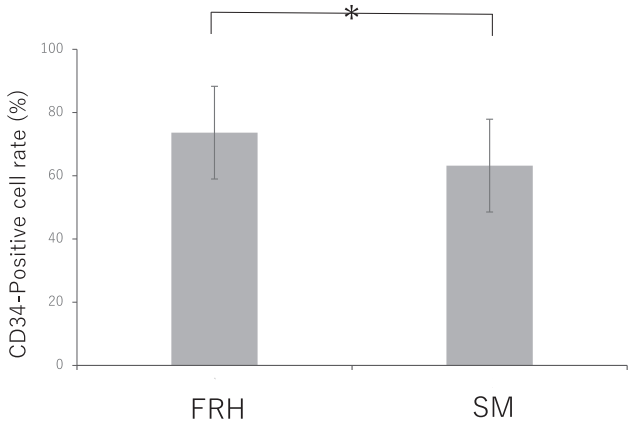

Fig. 3. Quantitative analysis of CD34-positive cells by immunohistochemistry. The CD34-positive cell rate is 63.21 ± 14.97% for healthy submucosa (SM) and 73.64 ± 14.36% for fibrous reactive hyperplasia (FRH). * $p < 0.05$.

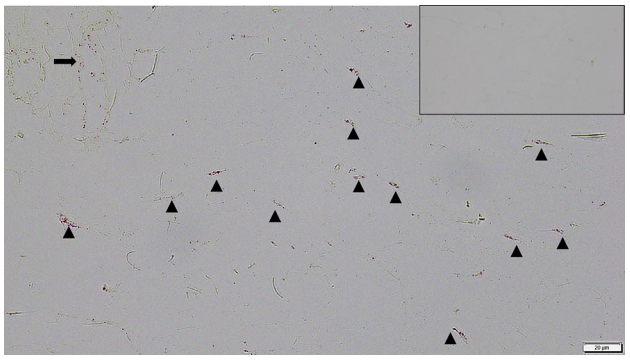

Fig. 4. RNA in situ hybridization. Red dots indicate *CD34*-positive mRNA signals. Vascular endothelial cells are used as a positive internal control and are confirmed to be positive for *CD34* mRNA (arrow). *CD34* expression is observed in the cytoplasm of spindle-shaped cells (arrowhead). The inset shows the section with no probe (Scale bar = 20 μm).

FRH tissues was 73.64 ± 14.36% (Fig. 3). Thus, FRH showed a significant increase in CD34-positive cells compared to those of healthy surrounding tissues ($p = 0.036$).

3.4. ISH for CD34 mRNA

CD34 expression was observed in the cytoplasm of spindle-shaped fibroblasts in FRH samples (Fig. 4). Vascular endothelial cells also showed positive *CD34* expression (Fig. 4, arrow).

3.5. Analysis of CD34 mRNA expression

CD34 mRNA expression was confirmed in all cases, including the palate. Furthermore, a correlation was found between the ratio of CD34-positive cells and the *CD34* mRNA copy number ($r = 0.823$, $p < 0.001$) (Fig. 5).

4. Discussion

In the buccal, tongue, palate, and lip mucosa, fibroblasts in the lamina propria of healthy oral mucosa did not express CD34. Manabe et al. [11] showed that CD34 was not expressed in the fibroblasts of gingival lamina propria. Furthermore, Roman et al. [15] reported that the only CD34-positive cells in the palate mucosa were vascular endothelial cells; no CD34-positive fibroblasts were found. However, in our study, CD34-positive cells were observed in abundance in the submucosal fibroblasts of buccal, tongue, and lip mucosa. Although CD34 has been reported to be ubiquitously expressed in fibroblasts of the dermis and connective tissue [16], fibroblasts in the lamina propria of the oral mucosa was negative for CD34 in the present study. This CD34 expression profile may indicate differences in the characteristics of fibrous connective tissues between the lamina propria and submucosa in the oral cavity.

Our results indicated that CD34-positive fibroblasts were present in submucosal connective tissues. FRH is located mainly in the submucosa under the lamina propria. Chen et al. [17] reported that mechanical stress on fibroblasts not only increased *CD34* mRNA

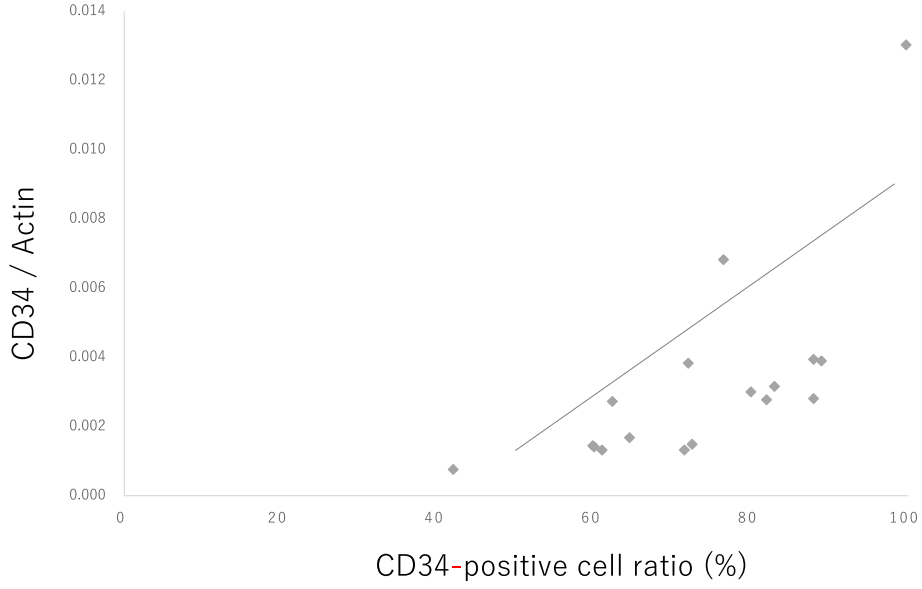

Fig. 5. The correlation between the CD34-positive cell rate and *CD34* mRNA copy number. We identified a positive linear correlation between the CD34-positive cell rate and the *CD34* mRNA copy number ($r = 0.823$, $p < 0.001$).

expression but also increased the synthesis of *types I* and *IV collagen* mRNA. FRH found in the mucosa of the buccal, tongue, and lips may have been caused by increased collagen synthesis in CD34-positive fibroblasts from the submucosal connective tissue. The submucosa contains fatty tissue and minor salivary glands and is more susceptible to external stimuli than the lamina propria because of its loose connective tissue [18]. Interestingly, the frequency of FRH is much lower in the palate than in the tongue or buccal mucosa [19]. Thus, increased collagen synthesis due to the activation of CD34-positive cells may play a role in protecting the submucosa from irritation. However, the two cases of FRH derived from the palate were CD34-negative. Consistent with our findings, Kinoshita et al. [20] reported that fibroblasts from irritated fibromas in the palate were CD34-negative. Manabe et al. [11] reported that fibrous epulides and peripheral odontogenic fibromas originating from the gingiva were also CD34-negative. FRH at sites without submucosal tissues, such as the gingiva and parts of the palate, may be derived from CD34-negative fibroblasts in the lamina propria; however, this must be validated in further studies.

Through quantitative PCR, positive *CD34* expression was detected in all cases of FRH. It is known that CD34 is not only expressed in fibroblasts but also in vascular endothelial cells [21]. *CD34* mRNA expression was also detected in FRH samples derived from the palate because they contained blood vessels. *CD34* mRNA signals were further detected through ISH in vascular endothelial cells and fibroblasts. To our knowledge, this is the first study reporting *CD34* mRNA expression in FRH tissues. In addition, we identified a positive correlation between the CD34-positive cell ratio and the *CD34* mRNA copy number. It has been reported that CD34 expression, in cooperation with CD90 expression in the fibroblastic network, was associated with collagenous scarring [22]. CD34-positive cells are known to be the most important component of EF [23] and SFT [24]; CD34-mediated signaling reportedly plays a role in the pathogenesis of EF [25]. Since signal transduction via CD34 may be involved in collagen synthesis in FRH, we believe that our analysis of *CD34* mRNA expression in FRH is important for future research.

In summary, our findings suggest that FRH is caused by an increase in collagen synthesis by CD34-positive fibroblasts in the submucosa. Furthermore, because mRNA and protein of CD34 was expressed in FRH, we intend to elucidate the mechanism of collagen synthesis mediated by CD34 in human submucosal fibroblasts in future studies.

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Conflict of interest

The authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

Masako Nagasaki: Data curation, Formal analysis, Investigation, Writing - original draft. **Wakako Sakaguchi:** Writing - review & editing, Investigation. **Keiichi Tsukinoki:** Conceptualization, Validation, Project administration, Writing - review & editing.

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References

[1] Toida M, Tatematsu N, Murakami T, Kato K, Kusunoki Y, Yasuda S, et al. Irritation fibroma of the oral mucosa: a clinicopathological study of 129 lesions in 124 cases. Oral Med Pathol 2001;6:91–4.
[2] Lee JH, An JS, Lee ES, Kwon SY, Kim YS. Comparison of sporadic sclerotic fibroma and solitary fibrous tumor in the oral cavity. Yonsei Med J 2007;48:535–9.
[3] Cox DP, Daniels T, Jordan RC. Solitary fibrous tumor of the head and neck. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:79–84.
[4] Jaafari-Ashkavandi Z, Shirazi MY, Assar S. Desmoplastic fibroblastoma in maxillary alveolar bone mimicking an odontogenic lesion: a novel case report with review of literature. Turk Patoloji Derg 2018;34:247–50.
[5] Lombardi T, Kuffer R, Samson J. Sporadic sclerotic fibroma of the oral soft tissues. Am J Dermatopathol 2005;27:370.
[6] Potter TJ, Summerlin DJ, Rodgers SF. Elastofibroma: the initial report in the oral mucosa. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:64–7.
[7] Díaz-Flores L, Gutiérrez R, García MP, Sáez FJ, Díaz-Flores Jr L, Valladares F, et al. CD34+ stromal cells/fibroblasts/fibrocytes/telocytes as a tissue reserve and a principal source of mesenchymal cells. Location, morphology, function and role in pathology. Histol Histopathol 2014;29:831–70.
[8] Geramizadeh B, Marzban M, Churg A. Role of immunohistochemistry in the diagnosis of solitary fibrous tumor, a review. Iran J Pathol 2016;11:195–203.
[9] Tardio JC. CD34-reactive tumors of the skin. An updated review of an ever-growing list of lesions. J Cutan Pathol 2009;36:89–102.
[10] Gun BD, Bahadir B, Behzatoglu K, Gun MO, Ozdamar SO. Elastofibroma: a clinicopathologic and immunohistochemical study of seven cases and literature review. APMIS 2007;115:115–9.
[11] Manabe K, Yakeishi M, Sakaguchi W, Saruta J, Tsukinoki K. Histopathological analysis of the differential diagnosis of peripheral odontogenic fibroma from fibrous epulis. J Oral Biosci 2019 Dec;61(4):221–5. https://doi.org/10.1016/j.job.2019.10.003. Epub 2019 Oct 25.
[12] Nakamura F, Fifita SF, Kurayama K. A study of oral fibroma with special reference to clinicopathological and immunohistochemical features of oral stromal spindle cells. Int J Oral-Med Sci 2005;4:83–91.
[13] O'Malley KJ, Eisermann K, Pascal LE, Parwani AV, Majima T, Graham L, et al. Proteomic analysis of patient tissue reveals PSA protein in the stroma of benign prostatic hyperplasia. Prostate 2014;74:892–900.
[14] Kang YG, Jung CK, Lee A, Kang WK, Oh ST, Kang CS. Prognostic significance of S100A4 mRNA and protein expression in colorectal cancer. J Surg Oncol 2012;105:119–24.
[15] Roman A, Páll E, Mihu CM, Petruțiu AS, Barbu-Tudoran L, Câmpian RS, et al. Tracing CD34+ stromal fibroblasts in palatal mucosa and periodontal granulation tissue as a possible cell reservoir for periodontal regeneration. Microsc Mircoanal 2015;21:837–48.
[16] Sidney LE, Branch MJ, Dunphy SE, Dua HS, Hopkinson A. Concise review: evidence for CD34 as a common marker for diverse progenitors. Stem Cell 2014;32:1380–9.
[17] Chen J, Zhang W, Backman LJ, Kelk P, Danielson P. Mechanical stress potentiates the differentiation of periodontal ligament stem cells into keratocytes. Br J Opthalmol 2018;102:562–9.
[18] Ten Cate AR, Nanci A. Ten cate's oral histology: development, structure, and function. Philadelphia: Elsevier; 2008.
[19] Barker DS, Lucas RB. Localised fibrous overgrowths of the oral mucosa. Br J Oral Surg 1967;5:86–92.
[20] Kinoshita H, Ogasawara T, Toya T, Makihara R, Hirai R, Kawahara E. Slow-growing large irritation fibroma of the anterior hard palate: a case report using immunohistochemical analysis. J Maxillofac Oral Surg 2016;15:253–7.
[21] Nonaka CFW, Rêgo DM, Miguel MCDC, De Souza LB, Pinto LP. Elastofibromatous change of the oral mucosa: case report and literature review. J Cutan Pathol 2010;37:1067–71.
[22] Ho JD, Chung HJ, Ms Barron A, Ho DA, Sahni D, Browning JL, et al. Extensive CD34-to-CD90 fibroblast transition defines regions of cutaneous reparative, hypertrophic, and keloidal scarring. Am J Dermatopathol 2019;41:16–28.
[23] Yamazaki K. An ultrastructural and immunohistochemical study of elastofibroma: CD 34, MEF-2, prominin 2 (CD133), and factor XIIIa-positive proliferating fibroblastic stromal cells connected by Cx43-type gap junctions. Ultrastruct Pathol 2007;31:209–19.
[24] Smith SC, Gooding WE, Elkins M, Patel RM, Harms PW, McDaniel AS, et al. Solitary fibrous tumors of the head and neck: a multi-institutional clinicopathologic study. Am J Surg Pathol 2017;41:1642–56.
[25] Hisaoka M, Hashimoto H. Elastofibroma: clonal fibrous proliferation with predominant CD34-positive cells. Virchows Arch 2006;448:195–9.