

Low-grade myofibroblastic sarcoma arising in the tip of the tongue with intravascular invasion: A case report

YURIE MIKAMI^{1,2}, SHINSUKE FUJII¹, KEN-ICHI KOHASHI³, YUICHI YAMADA³, MASAFUMI MORIYAMA², SHINTARO KAWANO², SEIJI NAKAMURA², YOSHINAO ODA³ and TAMOTSU KİYOSHIMA¹

¹Laboratory of Oral Pathology and ²Section of Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University; ³Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan

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Abstract. Low-grade myofibroblastic sarcoma (LGMS) is a rare intermediate tumor, which rarely metastasizes and has myofibroblastic differentiation in various sites. It is particularly associated with the tongue in the head and neck region. The lack of any pathological features means it is difficult to make a conclusive diagnosis of LGMS. The immunohistochemical features and genomic rearrangements, including *SS18-SSXs* and *MYH9-USP6s* and the genetic mutations of cancer-associated genes, including *APC*, *CTNNB1*, *EGFR*, *KRAS*, *PIK3CA* and *p53* were examined in a case of LGMS arising in the tip of the tongue. Immunohistochemically, the tumor cells were positive for alpha-smooth muscle actin and vimentin, as in previous reports. They demonstrated neither genomic rearrangements nor point mutations of cancer-associated genes. Although several tumor cells demonstrated intravascular invasion, the MIB-1 labeling index of the cells was the same as the original lesion. To the best of our knowledge, this is the first case report of LGMS arising in the tip of the tongue with intravascular invasion.

Introduction

Low-grade myofibroblastic sarcoma (LGMS) is a rare sarcoma tumor. Atypical myofibroblasts have been shown to be involved in LGMS, such as in the femur (1), vulva (2) and phalanx (3). In the head and neck region, LGMS frequently occurs in the tongue (4). Myofibroblasts, which show characteristics of both fibroblasts and smooth muscle cells, were

firstly identified in granulation tissue in 1971 (5). Fibroblasts differentiate into myofibroblasts during wound healing and tissue repair (6). According to the 2013 World Health Organization (WHO) classification (7), LGMS is categorized as a fibroblastic/myofibroblastic tumor; intermediate tumors (rarely metastasizing). Clinically, LGMS has a propensity for local recurrence and is associated with a low risk of metastatic spread. Immunohistochemically, LGMS tumor cells are positive for at least one myogenic marker, such as alpha-smooth muscle actin (α -SMA), desmin or muscle actin (HHF-35). As no definitive markers of LGMS have been found to date, it is difficult to make a diagnosis based on the pathological findings alone. In addition, whether or not the genetic mutations of cancer-related genes shown in sarcomas are involved in LGMS is unclear.

In the present study, we carried out immunohistochemical analyses in LGMS arising in the tip of the tongue in a sporadic case, examined genomic rearrangements, such as *SS18-SSXs* (*SS18-SSX1*, *SS18-SSX2*, *SS18-SSX4*, *SS18-SSX4V*, *SS18p-SSXp* or *SS18-SSXp*) and *MYH9-USP6s* (*MYH9* exon 1 and *USP6* exon 1 or *MYH9* exon 1 and *USP6* exon 2) for differential diagnostic supports and investigated whether or not these LGMS tumor cells harbored point mutations in the *APC*, *CTNNB1*, *EGFR*, *KRAS*, *PIK3CA* and *p53* genes for detection of the malignant tumor behavior.

Case report

A 38-year-old woman visited at the Department of Oral and Maxillofacial Surgery at Kyushu University Hospital (Fukuoka, Japan) with a 2-month history of a painless mass showing gradual growth in the tip of the tongue (Fig. 1A). An oral examination revealed that the tumorous mass was soft-elastic and well-circumscribed, and the overlying mucosal surface showed redness and a normal texture. There was no clinical evidence of cervical lymphadenopathy. She had a medical history of idiopathic thrombocytopenic purpura (cured) and fibroma of her right breast (under observation). An ultrasonogram examination revealed that the mass had a hypoechoic area with undetectable vascularization (Fig. 1B). Based on these findings, the tumorous mass was clinically diagnosed as not a malignant tumor. An excisional biopsy was performed (Fig. 1C and D),

Correspondence to: Professor Tamotsu Kiyoshima, Laboratory of Oral Pathology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
E-mail: kiyo@dent.kyushu-u.ac.jp

Key words: low-grade myofibroblastic sarcoma, intravascular invasion, genomic rearrangements, genetic mutations, tip of the tongue

Table I. Forward and reverse primers for genomic rearrangement analysis.

Gene	Primer sets	Product size (bp)
SS18-SSX1	F: 5'-AGACCAACACAGCCTGGACCAC-3' R: 5'-GGTGCAGTTGTTTCCCATCG-3'	151
SS18-SSX2	F: 5'-AGACCAACACAGCCTGGACCAC-3' R: 5'-GCACTTCCTCCGAATCATTTTC-3'	109
SS18-SSX4	F: 5'-AGACCAACACAGCCTGGACCAC-3' R: 5'-TCTGGCACTTCCTTCAAACC-3'	113
SS18-SSX4V	F: 5'-AGACCAACACAGCCTGGACCAC-3' R: 5'-CCAGCTGCTTTTCTTACGC-3'	119
SS18p-SSXp	F: 5'-CCAGCAGAGGCCTTATGGATA-3' R: 5'-TTTGTGGGCCAGATGCTTC-3'	98
SS18-SSXp	F: 5'-AGACCAACACAGCCTGGACCAC-3' R: 5'-TTTGTGGGCCAGATGCTTC-3'	130
MYH9 (exon 1)-USP6 (exon 1)	F: 5'-GGGGCAGATCCAGGTTTCAG-3' R: 5'-GAAACTGGGCATCTCTGTGGC-3'	120
MYH9 (exon 1)-USP6 (exon 2)	F: 5'-GGGGCAGATCCAGGTTTCAG-3' R: 5'-GATGGACATGGTAGAGAATGC-3'	122

Annealing for all polymerase chain reaction products was performed at 62°C. F, forward; R, reverse.

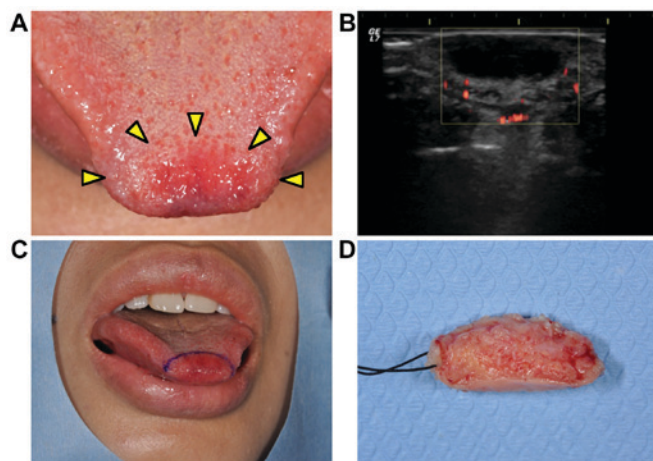


Figure 1. (A) A macroscopic photo of the tongue at the initial visit. The well-circumscribed elastic soft, 15x10x5 mm mass was overlaid with mucosa showing the redness of the surface. The yellow triangles indicate the boundary of the mass. (B) An ultrasonogram examination revealed that the mass was a 13x5 mm hypoechoic area with undetectable vascular flow. An excisional biopsy was performed. (C) The incision line indicates the resection of the tumorous mass with 2-3 mm normal tissue. (D) Following resection the surface was shown.

and the mass showed proliferation of spindle-shaped cells with fascicular and/or storiform patterns in the subepithelial nodular tumor lesion (Fig. 2A). Nuclear pleomorphism was shown in the tumor cells. The number of mitotic figures was <1 per 10 high-power fields (Fig. 2B).

Immunohistochemical examination was performed as described below. Paraformaldehyde-fixed paraffin-embedded (PFPE) tissues were cut at 4- μ m thickness. Immunohistochemical staining was performed using BondIII (Leica Microsystems GmbH, Wetzlar, Germany) according to the

manufacturer's instructions in the Division of Diagnostic Pathology in Kyushu University Hospital. The following monoclonal antibodies were used: Mouse anti-AE1/AE3 (1:100; cat. no. M 3515; Agilent Technologies, Inc., Santa Clara, CA, USA), mouse anti-EMA (1:100; cat. no. M 0613; Agilent Technologies, Inc.), mouse anti-CAM5.2 (1:100; cat. no. 349205; BD Biosciences, Franklin Lakes, NJ, USA), mouse anti- α -SMA (1:500; cat. no. M 0851; Agilent Technologies, Inc.), mouse anti-CD34 (1:1,000; cat. no. PA0212; Leica Biosystems, Wetzlar, Germany), mouse anti-MIB-1 (1:100; cat. no. M 7240; Agilent Technologies, Inc.), and mouse anti-vimentin (1:5; cat. no. 760-2512; Ventana Medical Systems, Inc., Tucson, AZ, USA). Immunohistochemical staining was also performed with the universal immunoperoxidase polymer method (Envision-kit; Agilent Technologies, Inc.) using monoclonal mouse anti-BAF47 (INI1 gene product) (1:250; cat. no. 612110; BD Biosciences) antibody. Immunohistochemical analyses showed that the spindle-shaped cells were positive for α -SMA (Fig. 2C) and vimentin (Fig. 2D) and negative for epithelial markers; AE1/AE3, EMA and CAM5.2 (data not shown). The MIB-1 (monoclonal antibody against Ki-67; cell proliferation marker) labeling index was 10% in the hot spot (Fig. 2E). Almost all spindle-shaped cells were positive for BAF47 similar to the cells in non-tumor tissues, such as inflammatory cells and endothelial cells (Fig. 2F).

In addition, genomic rearrangements, such as *SS18-SSXs* (*SS18-SSX1*, *SS18-SSX2*, *SS18-SSX4*, *SS18-SSX4V*, *SS18p-SSXp* or *SS18-SSXp*) and *MYH9-USP6s* (*MYH9* exon 1 and *USP6* exon 1 or *MYH9* exon 1 and *USP6* exon 2), for differential diagnostic supports, were not detected (Fig. 2G). Total RNA was extracted from PFPE sections with the RNeasy FFPE kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Five micrograms of RNA was reverse-transcribed using ReverTra Ace transcriptase (Toyobo

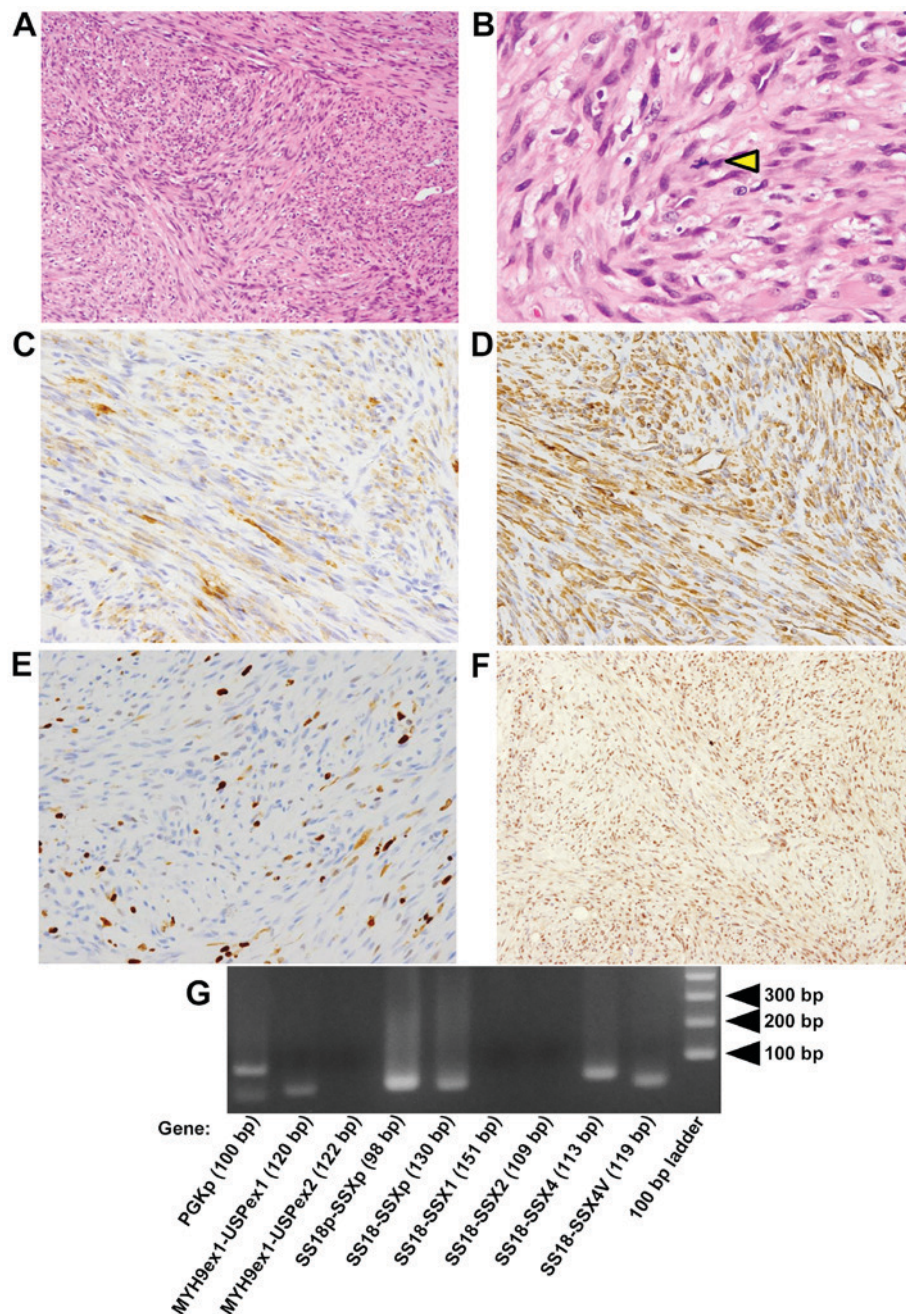


Figure 2. (A) Hematoxylin-eosin staining. (A) The proliferation of spindle-shaped cells with fascicular and/or storiform patterns is shown (magnification, x100). (B) The triangle indicates a mitotic figure (magnification, x400). The cytoplasm of the tumor cells was stained for (C) α-smooth muscle actin (magnification, x200) and (D) vimentin (magnification, x200). (E) Positivity for MIB-1 staining was noted in 10% of the tumor cells (magnification, x200). (F) Almost all the spindle cells were positive for BAF47 (magnification, x200). (G) The result of polymerase chain reaction.

Life Science, Osaka, Japan) in order to prepare the first-strand cDNA. Each polymerase chain reaction (PCR) product (10 μl) was directly loaded onto 2% agarose gel, stained with ethidium bromide, and directly visualized under UV illumination. Amplification of 100 base-pair (bp) products of PGKp was positive control of this PCR. Forward and reverse primers and annealing temperatures were summarized in Table I.

Furthermore, PCR amplification and sequencing analysis were carried out for assessing mutations. We extracted genomic DNAs from macrodissected PFPE sections using the WaxFree™ Paraffin Sample DNA Extraction kit (Trimgen, Sparks Glencoe, MD, USA) according to the manufacturer's instructions (8). The mutational status of *APC*, *CTNNB1* (9),

EGFR (10), *KRAS*, *PIK3CA* (11) and *p53* (12) genes was investigated using a PCR and direct sequencing, and no genetic mutations of these cancer-related genes were detectable. In Table II, forward and reverse primers for indicated exons in this sequencing analysis and annealing temperatures were shown. Copy number variations (CNVs) of the chromosomal intervals and/or candidate genes were not examined in the present study.

Several intravascular invasions of the tumor cells were found (Fig. 3A). Intravascular invading tumor cells were positive for vimentin (Fig. 3B). The vasculature was lined by CD34-positive vascular endothelial cells (Fig. 3C) and supported by α-SMA-positive (Fig. 3D) muscular tissue. Furthermore, elastic fibers were positive for Elastica van Gieson (EVG)

Table II. Forward and reverse primers for genetic mutation analysis of cancer-related genes.

Gene	Primers	Product size (bp)
APC-1-Exon 16	F: 5'-TAGGATGTAATCAGACGACAC-3' R: 5'-CAGTCTGCTGGATTGTTTC-3'	152
APC-2-Exon 16	F: 5'-GAAGTTCCAGCAGTGTCAACA-3' R: 5'-GTGTTTCAGGTGGACTTTTGG-3'	162
APC-3-Exon 16	F: 5'-CAAAAGTGGTGCTCAGACAC-3' R: 5'-TGCCACTTACCATTCCACTG-3'	164
APC-4-Exon 16	F: 5'-TCCGTTTCAGAGTGAACCATG-3' R: 5'-GGTACTTCTCGCTTGGTTTG-3'	149
APC-5-Exon 16	F: 5'-GTAAAACACCTCCACCACCT-3' R: 5'-TCAGCATCTGGAAGAACCTG-3'	151
APC-6-Exon 16	F: 5'-AATGCTGCAGTTCAGAGGGT-3' R: 5'-CCTGAACTGGAGGCATTATTC-3'	172
APC-7-Exon 16	F: 5'-CTCGATGAGCCATTATACAG-3' R: 5'-AGGTCCTTTTCAGAATCAATAG-3'	158
CTNNB1-Exon 3	F: 5'-GAAAAGCGGCTGTTAGTCAC-3' R: 5'-GAGAAAATCCCTGTTCCAC-3'	133
EGFR-Exon 18	F: 5'-TGTCTCTGTGTTCTTGTCCC-3' R: 5'-CCAGGGACCTTACCTTATAC-3'	162
EGFR-Exon 19	F: 5'-GCCAGTTAACGTCTTCCTTC-3' R: 5'-CCACACAGCAAAGCAGAAAC-3'	157
KRAS-Exon 1	F: 5'-GGTACTGGTGGAGTATTTGA-3' R: 5'-CAAGATTTACCTCTATTGTTGG-3'	158
PIK3CA-Exon 20	F: 5'-AACTGAGCAAGAGGCTTTGG-3' R: 5'-CTTTTCAGTTCAATGCATGCTG-3'	122
p53-Exon 5	F: 5'-CTCTTCTGCTGAGTACTCCCCTGC-3' R: 5'-GCCCCAGCTGCTCACCATCGCTA-3'	211
p53-Exon 6	F: 5'-GTGCAGCTGTGGGTTGATT-3' R: 5'-GGCCACTGACAACCACCCTTAACC-3'	182
p53-Exon 7	F: 5'-GCTTGCCACAGGTCTCCCCAAG-3' R: 5'-AGGCTGGCAAGTGGCTCCTGAC-3'	192
p53-Exon 8	F: 5'-TGGTAATCTACTGGGACGGA-3' R: 5'-GCTTAGTGCTCCCTGGGGGC-3'	134
p53-Exon 9	F: 5'-GCCTCTTTCCTAGCACTGCCCAAC-3' R: 5'-CCCAAGACTTAGTACCTGAAGGGTG-3'	102

Annealing for all polymerase chain reaction products was performed at 60°C, except for EGFR at 59°C, KRAS at 59°C and PIK3CA at 57°C. F, forward; R, reverse.

staining (Fig. 3E). EVG staining was performed according to the standard protocol. The MIB-1 labeling index of the tumor cells noted in the vascular tissue was 2-10% (Fig. 3F). Since the histological findings showed a tumor-free region with 2-3 mm normal tissue in the extirpated sample, re-resection of the tongue was performed to obtain a tumor-free region with 10 mm normal tissue. The patient had no evidence of local recurrence or metastasis in the two and a half years since the first operation.

Discussion

LGMS is known to be a rare sarcoma tumor (1-3). In the head and neck region, LGMS frequently occurs in the tongue, especially in the lateral edge of the tongue (13) compared with that

in the skin and gastrointestinal tract. To our knowledge, this is the first report of LGMS arising in the tip of the tongue. In the 2013 WHO classification, LGMS is an intermediate-rarely metastasizing tumor mainly composed of atypical myofibroblasts. Myofibroblasts share immunohistochemical and ultrastructural features of both fibroblasts and smooth muscle cells and play a role in wound and tissue repair (14). However, the function of myofibroblasts in LGMS has been unclear. LGMS tends to be found in local recurrence rather than metastasis (15). The current case showed a low MIB-1 labeling index in the tumor cells and several intravascular invasions of the tumor cells. The MIB-1 labeling index of the intravascular tumor cells was 2-10%, which was almost the same as that in the original tumor (Fig. 3E). There has been no evidence of

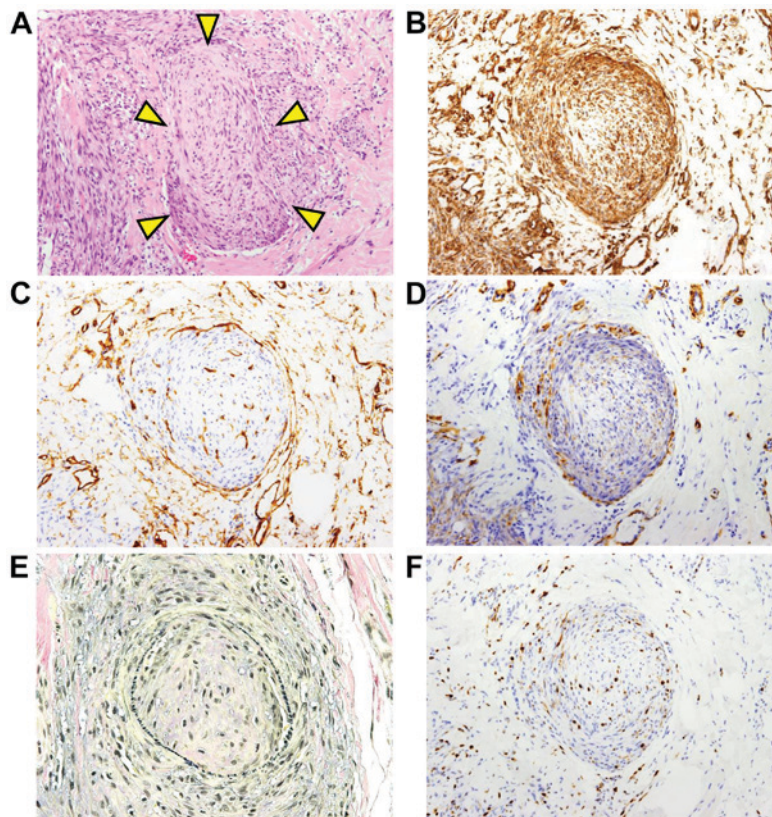


Figure 3. Intravascular invasion of LGMS tumor cells. (A) The triangles indicate the tumor cells invading into the vascular tissue. Hematoxylin-eosin staining (magnification, x100). (B) The intravascular invading tumor cells were stained with vimentin (magnification, x100). (C) The vascular endothelial cells were stained for cluster of differentiation 34 (magnification, x100). (D) The vasculature was surrounded by α -smooth muscle actin-positive muscular tissue (magnification, x100). (E) The vascular elastic fibers were stained for Elastica van Gieson (magnification, x100). (F) The intravascular invading tumor cells were stained with MIB-1 antibody (magnification, x100). LGMS, low-grade myofibroblastic sarcoma.

local recurrence or metastasis in the two and a half years since the first operation. Therefore, these findings were consistent with the description by the WHO that LGMS has a malignant potential and is an intermediate-rarely metastasizing tumor.

The differential diagnosis of LGMS should include other spindle cell tumors, such as benign tumors; inflammatory myofibroblastic tumors and nodular fasciitis; such as intermediate tumor; desmoplastic fibroma or malignant tumors, such as synovial sarcoma (12), leiomyosarcoma and osteosarcoma (1). Inflammatory myofibroblastic tumor shows infiltration of inflammatory cells. As the genomic rearrangement of *MYH9-USP6s* (*MYH9* exon 1 and *USP6* exon 1 or *MYH9* exon 1 and *USP6* exon 2), which is frequently observed in nodular fasciitis (16), was not shown in the current case (data not shown), nodular fasciitis would be excluded. Synovial sarcoma would be ruled out, because reduced expression of BAF47 (INI1 gene product) protein (17), which is shown in synovial sarcoma, was not observed. Neither rhabdoid cells, which are characterized by the existence of a large eosinophilic inclusion within the cytoplasm, eccentric nuclei and prominent nucleoli, nor the genomic rearrangement of *SS18-SSXs* (*SS18-SSX1*, *SS18-SSX2*, *SS18-SSX4*, *SS18-SSX4V*, *SS18p-SSXp* or *SS18-SSXp*) were detected in the case (Fig. 2G). In general, malignant tumor cells frequently show increased number of mitotic figures and point mutations of cancer-related genes. The tumor cells involved in the current LGMS showed no point mutations in the *APC*, *CTNNT1*, *EGFR*, *KRAS*, *PIK3CA* or *p53* gene (data not shown). Many cases of

leiomyosarcoma (18) and osteosarcoma (19) have been reported to have a gene mutation in *p53*, and desmoplastic fibroma has a point mutation in *CTNNT1*. Therefore, point mutations of the investigated genes might not be involved in intravascular invasion of myofibroblasts in LGMS. Because there are no established treatments for LGMS at present, the mechanism underlying the invasion of atypical myofibroblasts into vascular tissue should be clarified in the future.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Author contributions

YM performed the immunohistochemical experiments. YM and SF designed and wrote the manuscript. YM, MM, SK,

and SN performed the clinical diagnosis, treatment (including resection of the tongue) and follow-up of the patient. KK, YY and YO performed the mutations and genomic rearrangements analysis. YM, SF, KK, YY, YO and KT made the histopathological diagnosis. KT interpreted results and co-wrote the manuscript.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Kyushu University (approval nos. 25-111, 26-257, 27-77 and 29-625). Written informed consent was obtained from the patient.

Patient consent for publication

Written informed consent was obtained from the patient for the publication of their data.

Competing interests

The authors declare that they have no competing interests.

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