β-catenin (*CTNNB1*) Mutation and LEF1 Expression in Sinonasal Glomangiopericytoma (Sinonasal-type Hemangiopericytoma)

Yuka Suzuki[1], Shu Ichihara[2], Tomonori Kawasaki[2], Hiroyuki Yanai[3], Satoshi Kitagawa[4], Yoshie Shimoyama[1], Shigeo Nakamura[1], Masato Nakaguro[1],

[1]Department of Pathology and Laboratory Medicine, Nagoya University Hospital, Nagoya, Japan; [2]Department of Pathology, Nagoya Medical Center, Nagoya, Japan; [3]Department of Pathology, Okayama University Hospital, Okayama, Japan; [4]Department of Pathology, Toyota Memorial Hospital, Toyota, Japan

Corresponding author:

Masato Nakaguro, M.D., Ph.D
Department of Pathology and Clinical Laboratories,
Nagoya University Hospital,
65 Tsurumai, showa-ku, Nagoya, 466-8560, Japan
Phone, +81-52-744-2648
FAX, +81-52-744-2651
E-mail: mnakaguro@med nagoya-u.ac.jp
ORCID: 0000-0001-6987-3043

Key words: Sinonasal glomangiopericytoma, β-catenin, *CTNNB1*, LEF1

Word count: total 1441 words

Abstract

Sinonasal glomangiopericytoma (SN-GPC) is an uncommon mesenchymal tumor with myoid differentiation. Recently, mutations in exon 3 of the gene coding for β-catenin (*CTNNB1*) and its nuclear expression were discovered in SN-GPC. β-catenin protein is a key regulatory molecule of the canonical Wnt signaling pathway. The expression of β-catenin target proteins is not well characterized in SN-GPC. We examined 3 SN-GPCs by immunohistochemistry and *CTNNB1* mutation analysis. All cases expressed nuclear β-catenin. We identified *CTNNB1* exon 3 mutations in 2 analyzable cases. Lymphoid enhancer binding factor 1 (LEF1), a protein downstream from β-catenin, was also expressed in all cases. Our results further characterized the activation of the Wnt signaling pathway caused by *CTNNB1* exon 3 mutation and suggest the utility of LEF1 immunohistochemistry in the differential diagnosis of SN-GPC.

Introduction

Sinonasal glomangiopericytoma (SN-GPC) is a rare sinonasal mesenchymal neoplasm. Originally, this tumor was reported as a "hemangiopericytoma-like intranasal tumor" [1], and thereafter its distinctive nature and its cellular origin have been discussed extensively [2, 3]. SN-GPC is characterized by diffuse growth of bland-looking ovoid or spindle monotonous tumor cells. In contrast to solitary fibrous tumors (SFT, formerly designated hemangiopericytoma), SN-GPC shows a myoid phenotype demonstrated by expression of α-smooth muscle actin (α-SMA) and a lack of CD34 or STAT6 expression [2, 4].

β-catenin (encoded by the *CTNNB1* gene) is a key regulatory molecule of the canonical Wnt signaling pathway. An activating mutation in exon 3 of *CTNNB1* causes accumulation of β-catenin in the nucleus and activates the transcription of downstream target genes such as c-Myc (*MYC*), cyclin D1 (*CCND1*) and Lymphoid enhancer binding factor 1 (*LEF1*) [5]. Recently, nuclear β-catenin expression and *CTNNB1* exon 3 mutation were reported in SN-GPC [6, 7]. LEF1 protein is a transcription factor that has been implicated in the pathogenesis of multiple tumors. The utility of LEF1 immunohistochemistry has been demonstrated in salivary gland tumors or desmoid fibromatosis [8, 9]. The expression of LEF1 in SN-GPC has not been previously investigated. In this study, we describe 3 cases of SN-GPC in which we subjected tumors to immunohistochemical analysis of the Wnt signaling pathway and *CTNNB1* gene mutation analysis.

Material and Methods

Tissue samples

Three cases of SN-GPC were retrieved from our collection of institutional cases. Five cases of glomus tumors in fingers and five cases of SFT in soft tissue were also selected from institutional cases for comparison. Paraffin sections of tumor tissue samples were stained with hematoxylin and eosin.

Immunohistochemistry

Slides from each tumor were stained with a panel of commercially available primary antibodies, including antibodies against α-SMA (1A4; 1:1000 dilution; DakoCytomation), β-catenin (17C2; 1:400; Leica), LEF1 (EPR2029Y; 1:100; Abcam), CD34 (QBEnd-10; 1:600; Dako), STAT6 (YE361; 1:1000; Abcam), cyclinD1 (SP4; 1:100; Abcam), c-Myc (Y69; 1:50; Abcam) and Ki-67 (MIB-1; 1:500; Dako). For β-catenin, LEF1 and STAT6, only nuclear staining was accepted as an indication of positive expression. Positive β-catenin was defined as the staining of >90% of the tumor cells.

Mutation analysis

To detect *CTNNB1* exon 3 mutations, we performed polymerase chain reaction (PCR) assays followed by Sanger sequencing. DNA from formalin-fixed paraffin-embedded tissue was extracted with the QIAamp DNA FFPE Tissue Kit (QIAGEN). Exon 3 of *CTNNB1* was amplified using the following primer pair: forward, 5'-TTTGATGGAGTTGGACATGG -3' and reverse, 5'- AAAATCCCTGTTCCCACTCA –3'. The PCR reaction was performed with KOD FX enzyme (Toyobo). PCR products were electrophoresed and DNAs were extracted with NucleoSpin Gel and PCR Clean-up (Macherey-Nagel). Each purified product was directly sequenced using a CTNNB1-F primer with BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and 3730xl DNA Analyzer (Thermo

Fisher Scientific).

Results

Clinical features

The characteristics of the 3 SN-GPC patients are summarized in Table 1. Patients were 62- to 81-years-old (mean, 74.7) and included 1 male and 2 females. One patient had involvement in the right nasal cavity, whereas two were on the left side. All of the patients presented with nasal bleeding and were clinically diagnosed with hemangiomas or nasal polyps. All the cases were treated with simple tumorectomy. The follow-up periods were 4 and 14 months in 2 of the cases. For the remaining case (case 2), we could not obtain detailed clinical information. No local recurrences or metastases were reported.

Histological features

The 3 tumors showed similar histological features. The tumors were localized beneath the normal ciliated mucosa and demonstrated pushing borders without fibrous capsule formation (Fig. 1a). The tumors were rich in vasculature and intratumoral hemorrhage was sometimes observed. The blood vessel walls were thin and partially hyalinized (Fig. 1b). Most of the tumor cells had oval and, in limited areas, spindle-shaped nuclei with coarse chromatin and inconspicuous nucleoli (Fig. 1c). The nuclei were small-sized and nuclear atypia was minimal. No mitosis was observed. The cytoplasm was eosinophilic or partially clear with indistinct cell borders (Fig. 1d). Tumor cells grew with a fascicular, sheet-like or solid pattern. Bone destruction was observed in one case (case 3, Fig. 1e).

Immunohistochemical features

All cases were positive for α-SMA (Fig. 2a) and strongly expressed nuclear β-catenin in diffuse patterns (Fig. 2b). All 3 patients also expressed LEF1 in the nucleus (Fig. 2c). β-catenin and LEF1 expression was observed specifically in tumor

cells. All cases were negative for CD34 and STAT6 expression, both of which are diagnostic markers of SFT (Fig. 2d). The Ki-67 labeling index was no more than 2%. c-Myc and cyclinD1 were limited to partial and weak expression (data not shown). Two of 5 SFT cases were partially positive for LEF1 and all 5 cases were diffusely and strongly positive for STAT6 (Fig. 2e, f, Table 2). Five glomus tumors were negative for LEF1 and STAT6 (Table 2).

Mutation analysis

CTNNB1 exon 3 mutation analysis was successfully performed for 2 cases. Single bp missense mutations were detected in codon 35 (p.I35T, c.104T>C) in 1 cases and codon 41 (p.T41A, c.121A>G) in another case (Fig. 2g). Both kinds of mutation were included in previous reports [6, 7]. The more common types of mutation, mutations in codons 32, 33, 37 or 45, were not detected in our cases. In 1 case (case 2), PCR amplification was not successful due to DNA degradation caused by non-buffered formalin fixation.

Discussion

In 1976, Compagno et al. reported 23 cases of SN-GPC as “hemangiopericytoma-like intranasal tumors” and since then various terms have been given to this tumor [1]. In the newest WHO classification of head and neck tumors, the term SN-GPC was adopted [10]. SN-GPC is a clinically indolent tumor with SFT-like (formerly termed “hemangiopericytoma”) morphology and myoid phenotype. The myoid phenotype has been demonstrated by immunohistochemistry (positivity for α-SMA) or ultrastructure. Since SFT tumors do not show a myoid phenotype, a kinship of SN-GPC with glomus tumors rather than SFTs has been suggested [2].

In addition to its histological and immunohistochemical features, recent data showed additional characteristics of SN-GPC, including nuclear β-catenin expression and *CTNNB1* exon 3 mutations [6, 7]. In the canonical Wnt signaling pathway, β-catenin activates nuclear transcription factors such as cyclin D1 or LEF1. *CTNNB1* exon 3 mutations dysregulate the phosphorylation and degradation pathway of β-catenin and cause nuclear accumulation of β-catenin. Most of the reported cases are positive for nuclear β-catenin and harbor *CTNNB1* exon 3 mutations. In our study, 2 out of 3 cases were diffusely and strongly positive for nuclear β-catenin and harbored a *CTNNB1* exon 3 mutation. As the staining intensity of β-catenin varies between cases and tumor areas [11], immunohistochemical staining of β-catenin is sometimes difficult to evaluate. In desmoid fibromatosis, another *CTNNB1* mutation-related tumor, some cases lack β-catenin expression despite the presence of a *CTNNB1* mutation [11]. There is at least 1 report of SN-GPCs that were negative for β-catenin expression [12].

To examine possible alternative immunohistochemical markers for β-catenin, we performed immunohistochemical analyses for c-Myc, cyclin D1 and LEF1, all of which are downstream from β-catenin. Although a

previous report suggested the utility of cyclin D1 as a histochemical marker [6], cyclinD1 and c-Myc expression were partial and weak in our series. Diffuse LEF1 expression was observed specifically in tumor cells. A discrepancy in the expression of β-catenin and LEF-1 has been observed previously and the superior expression of LEF1 has led to the reported utility of LEF1 immunohistochemistry in desmoid fibromatosis or basaloid salivary gland tumors [8, 9].

We compared LEF1 expression by SN-GPCs to other sinonasal mesenchymal tumors. Glomus tumors can mimic SN-GPCs because of their similar cytological features and myoid marker expression [2, 13]. Unlike SN-GPCs, glomus tumors were completely negative for LEF1. Thus, LEF1 is a useful marker for the differentiation of SN-GPCs from glomus tumors. Although SFT tumors are composed of cells with more elongated or spindle-shaped nuclei, they show a proliferation pattern similar to SN-GPCs. Two out of 5 SFTs were positive for LEF1. As nuclear β-catenin expression is also observed in some SFTs despite the absence of a *CTNNB1* mutation [12], β-catenin and LEF1 immunohistochemistry are not useful in the exclusion of SFTs. Fortunately, SFTs are characterized by strong STAT6 positivity with much higher sensitivity and specificity [14]. In our series, as previously reported, all SN-GPCs were negative for STAT6 immunohistochemistry (Fig. 2e) [4]. Juvenile nasopharyngeal angiofibroma (JNA) also harbors *CTNNB1* mutations. JNA is less cellular and negative for lineage-specific myofibroblastic and smooth muscle markers [15]. Furthermore, JNA arises exclusively in adolescent male.

In conclusion, we have characterized 3 additional cases of SN-GPC and included *CTNNB1* exon 3 mutation analysis. All analyzable cases harbored a *CTNNB1* exon 3 mutation and expressed LEF1 specifically in tumor cells.

Acknowledgement

This study was supported, in part, by a Grant-in-Aid for Clinical Research from the National Hospital Organization.

Compliance with Ethical Standards

The study was approved by the institutional Review board.

Funding

No external funds were obtained for this work.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Compagno J, Hyams VJ (1976) Hemangiopericytoma-like intranasal tumors. A clinicopathologic study of 23 cases American journal of clinical pathology 66:672-683
2. Thompson LD, Miettinen M, Wenig BM (2003) Sinonasal-type hemangiopericytoma: a clinicopathologic and immunophenotypic analysis of 104 cases showing perivascular myoid differentiation Am J Surg Pathol 27:737-749
3. Granter SR, Badizadegan K, Fletcher CD (1998) Myofibromatosis in adults, glomangiopericytoma, and myopericytoma: a spectrum of tumors showing perivascular myoid differentiation The American journal of surgical pathology 22:513-525
4. Agaimy A, Barthelmess S, Geddert H, Boltze C, Moskalev EA, Koch M, Wiemann S, Hartmann A, Haller F (2014) Phenotypical and molecular distinctness of sinonasal haemangiopericytoma compared to solitary fibrous tumour of the sinonasal tract Histopathology 65:667-673. doi: 10.1111/his.12452
5. Taketo MM (2004) Shutting down Wnt signal-activated cancer Nature genetics 36:320-322. doi: 10.1038/ng0404-320
6. Lasota J, Felisiak-Golabek A, Aly FZ, Wang ZF, Thompson LD, Miettinen M (2015) Nuclear expression and gain-of-function beta-catenin mutation in glomangiopericytoma (sinonasal-type hemangiopericytoma): insight into pathogenesis and a diagnostic marker Mod Pathol 28:715-720. doi: 10.1038/modpathol.2014.161
7. Haller F, Bieg M, Moskalev EA, Barthelmess S, Geddert H, Boltze C, Diessl N, Braumandl K, Brors B, Iro H, Hartmann A, Wiemann S, Agaimy A (2015) Recurrent mutations within the amino-terminal region of beta-catenin are probable key molecular driver events in sinonasal hemangiopericytoma The American journal of pathology 185:563-571. doi: 10.1016/j.ajpath.2014.10.019
8. Zou Y, Zhang Y, Church J, Liu X (2016) Comparison of beta-Catenin and LEF1 Immunohistochemical Stains in Desmoid-type Fibromatosis and its Selected Mimickers, With Unexpected Finding of LEF1 Positivity in Scars Appl Immunohistochem Mol Morphol. doi: 10.1097/pai.0000000000000487
9. Bilodeau EA, Acquafondata M, Barnes EL, Seethala RR (2015) A comparative analysis of LEF-1 in odontogenic and salivary tumors Human pathology 46:255-259. doi: 10.1016/j.humpath.2014.10.018
10. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ WHO classification of head and neck tumours.
11. Huss S, Nehles J, Binot E, Wardelmann E, Mittler J, Kleine MA, Kunstlinger H, Hartmann W, Hohenberger P, Merkelbach-Bruse S, Buettner R, Schildhaus HU (2013) beta-catenin (CTNNB1) mutations and clinicopathological features of mesenteric desmoid-type fibromatosis Histopathology 62:294-304. doi: 10.1111/j.1365-2559.2012.04355.x

12. Jo VY, Fletcher CDM (2017) Nuclear beta-Catenin Expression is Frequent in Sinonasal Hemangiopericytoma and Its Mimics Head Neck Pathol 11:119-123. doi: 10.1007/s12105-016-0737-2

13. Tse LL, Chan JK (2002) Sinonasal haemangiopericytoma-like tumour: a sinonasal glomus tumour or a haemangiopericytoma? Histopathology 40:510-517

14. Doyle LA, Vivero M, Fletcher CD, Mertens F, Hornick JL (2014) Nuclear expression of STAT6 distinguishes solitary fibrous tumor from histologic mimics Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 27:390-395. doi: 10.1038/modpathol.2013.164

15. Pauli J, Gundelach R, Vanelli-Rees A, Rees G, Campbell C, Dubey S, Perry C (2008) Juvenile nasopharyngeal angiofibroma: an immunohistochemical characterisation of the stromal cell Pathology 40:396-400. doi: 10.1080/00313020802035857

Figure Legends

Fig. 1 Histological features of SN-GPC. (a) Tumor cells proliferate in the lamina propria to the submucosa with fascicular or sheet-like patterns. The surface ciliated mucosa is normal. (case 1, x200). (b) The tumor has a rich vasculature. In some areas, the blood vessel walls are hyalinized (case 3, x200). (c) The tumor cells have oval nuclei and the cytoplasms are eosinophilic (case 1, x400). (d) Clear cytoplasm is observed in a limited area (case 2, x400). (e) Bone destruction is observed in case 3 (x400).

Fig. 2 Immunohistochemical staining and *CTNNB1* exon 3 mutation analysis. (a-d) Immunohistochemical features of SN-GPC (case 3). Tumor cells express α-SMA (a), β-catenin (b) and LEF1 (c). β-catenin and LEF1 are expressed in tumor cell nuclei. (e,f) Immunohistochemical features of SFT. Three out of 5 SFTs were negative for LEF1 and 2 were positive (e, inset). All SFT cases expressed STAT6 in a diffuse and strong fashion (f). (g) Single bp missense mutations were detected in codon 41 (case 1) and codon 35 (cases 3).

					Immunohistochemistry						*CTNNB1* exon 3 mutation	
case	age	sex	follow up (mo.)	recurrence	α-SMA	β-catenin	LEF1	CD34	STAT6	Ki-67		
1	81	M	14	-	+	+	+	-	-	<1%	p.T41A	c.121A>G
2	62	F	unknown	unknown	+	+	+	-	-	1%	not analyzable	
3	81	F	4	-	+	+	+	-	-	2%	p.I35T	c.104T>C

Table 1. Clinical, immunohistochemical and molecular features of the 3 SN-GPCs

	LEF1	STAT6
GPC	3/3 (100%)	0/3 (0%)
SFT	2/5 (40%)	5/5 (100%)
GT	0/5 (0%)	0/5 (0%)

Table 2. Results of immunohistochemical analysis of LEF1 and STAT6 in SN-GPCs, SFTs and glomus tumors.

GPC = sinonasal glomangiopericytoma, HPC = solitary fibrous tumor, GT = glomus tumor

Figure1

Click here to download Figure 180411 Figure 1..tif

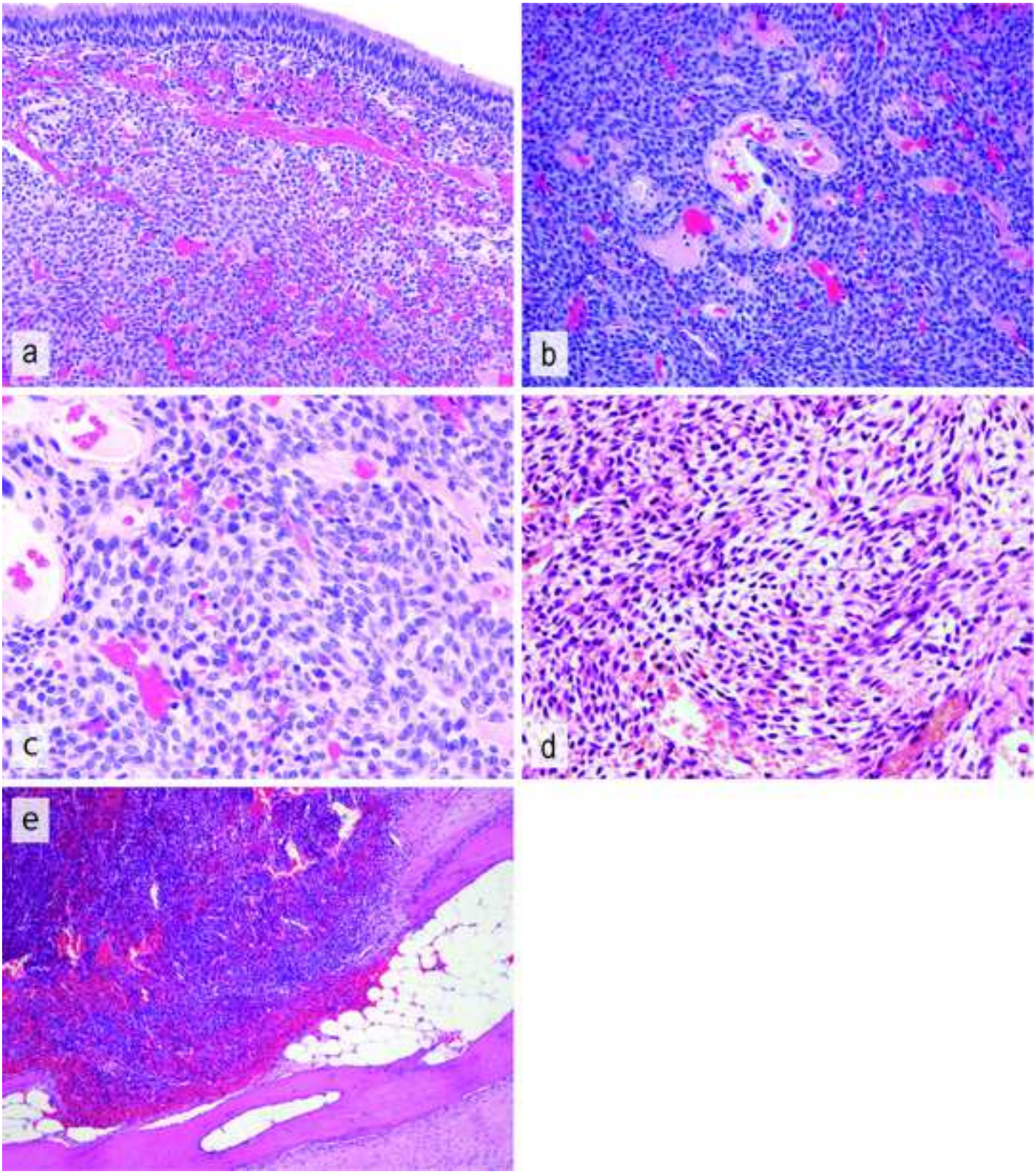

Figure2

Click here to download Figure 180430 Figure 2..tif