Original Article

Cytological Features of Mammary Analogue Secretory Carcinoma of Salivary Gland: Fine-Needle Aspiration of Seven Cases

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Abstract

Background: Mammary analogue secretory carcinoma (MASC) is a recently described salivary gland neoplasm that is defined by *ETV6-NTRK3* gene fusion. There have been few case reports on the cytopathologic features of MASC to date.

Methods: We examined the clinicopathological and cytological features of 7 cases of MASC defined by RT-PCR analysis of the *ETV6-NTRK3* fusion gene.

Results: The cases occurred in 3 men and 4 women aged between 39 and 68 years, with a mean of 51.6 years. In 5 of these 7 cases, the tumor involved the parotid gland. Histologically, all cases displayed predominantly microcystic patterns, often a mixture of follicular and papillary-cystic structures. All tumors were immunoreactive for mammaglobin, S-100 protein and vimentin. Available fine-needle aspiration cytology smears were cellular and exhibited many loosely cohesive syncytial clusters or isolated cells. Many histiocytes, some of which contained hemosiderin pigments, and variously shaped mucinous material were evident in the background or within the epithelial clusters. The majority of cases showed small to medium-sized follicular structures with secreted materials. Papillary clusters were occasionally found. Tumor cells exhibited small to medium-sized round to oval nuclei, with a smooth contour and indistinct or small nucleoli, and vacuolated cytoplasm. No tumor cells had obvious intracytoplasmic zymogen granules.

Conclusion: It appeared that clusters of small to medium-sized follicular and papillary configurations consisting of bland tumor cells with vacuolated cytoplasm, but lack of intracytoplasmic zymogen granules, in a mucinous or hemosiderin-laden histiocyte-rich background, were a characteristic cytological feature highly suggestive of MASC.

Key words: Salivary gland, mammary analogue secretory carcinoma, acinic cell carcinoma, aspiration cytology

Introduction

Mammary analogue secretory carcinoma (MASC) is a recently described salivary gland neoplasm.[1-4] It is defined by the t(12;15)(q13;q25) translocation that results in *ETV6-NTRK3* gene fusion identical to that found in secretory breast carcinoma.[5] The fusion gene, *ETV6-NTRK3*, encodes chimeric tyrosine kinase that has been shown to have transformative activity in epithelial and myoepithelial cells of the mouse mammary gland.[6] Histologically, MASC is characterized by the proliferation of uniform cells with vacuolated cytoplasm forming a microcystic, macrocystic and papillary architecture, which indicates the secretory functions of tumor cells. These histologic features overlap considerably with those of other salivary gland neoplasms, especially so-called acinic cell carcinoma, but MASC does not exhibit overt serous acinar differentiation by means of the presence of intracytoplasmic zymogen granules.[1-4]

Preoperative fine-needle aspiration cytology is a useful tool for triaging patients to appropriate management for salivary gland tumors.[7-9] This diagnostic procedure for salivary gland tumors, however, can be challenging. Factors causing diagnostic difficulties in the cytopathologic evaluation of salivary gland neoplasms include diverse cytomorphology and the infrequency of many tumor types, resulting in limited experience and lack of familiarity among cytotechnologists and pathologists. The salivary gland tumors are unique in their histological complexity and morphologic variability, which is reflected in the cytological material. While architecture and circumscription are frequently helpful in the histopathological differential diagnosis of salivary gland neoplasms, these clues are usually lacking in cytological preparations.

In general, cytological diagnostic criteria should be established whenever new histopathological entities happen to be recognized. As for MASC, however, there are

currently only a few case reports in the literature that describe the cytological features.[10-14] Therefore, the cytological characteristics of MASC are still not fully understood. Here, we present the cytological findings of 7 cases of molecularly proven MASC, and attempt to discuss their histological correlations and the cytological differential diagnosis of this newly recognized neoplasm from other well-known salivary gland tumors.

Materials and Methods

We searched for major salivary gland tumors previously diagnosed as acinic cell carcinoma deposited in our hospitals using RT-PCR for the detection of *ETV6-NTRK3* fusion gene, and then found 15 cases that were proven to be MASC. Of these, 7 cases were available for fine-needle aspiration smears and were analyzed for their cytologic features as well as the clinicopathological findings.

The aspirates were smeared onto glass slides and fixed in 95% ethanol for Papanicolaou stain and/or air-dried for May-Giemsa stain. The surgical specimen was fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin, Alcian blue, periodic acid-Schiff (PAS) and diastase-PAS.

Immunohistochemistry was performed on a Ventana BenchMark XT automated staining instrument according to the manufacturer's instructions. The primary antibodies used in this study are listed in Table 1. The antigen-antibody reaction was visualized with the chromogen 3,3′-diaminobenzidine. The sections were lightly counterstained with hematoxylin.

For RT-PCR, RNA was extracted from the formalin-fixed paraffin-embedded tissue and amplified as previously described.[15] The cDNA samples were then subjected

to PCR using the sense primer TEL971 (complementary to ETV6 with sequence 5′-ACCACATCATGGTCTCTGTCTCCC-3′) and the antisense primer TRKC1059 (complementary to NTRK3 with sequence 5′-CAGTTCTCGCTTCAGCACGATG-3′). The detection of a 110-bp fragment of *ETV6-NTRK3* fusion transcript was carried out according to the method described by Bourgeois et al.[16] In all positive cases, the breakpoints were confirmed by direct sequencing. The PCR fragments were directly sequenced by cycle sequencing with dye-labeled terminators (BigDye Terminators, Applied Biosystems) and analyzed on a DNA sequencer (model 310, Applied Biosystems).

Results

Clinicopathological Findings

The clinicopathological data are summarized in Table 2. The cases involved 3 men and 4 women aged between 39 and 68 years, with a mean of 51.6 years. The tumor involved the parotid gland in 5 patients, the accessory parotid gland in one and the submandibular gland in one. Six of them had an operation only, but one patient received post-operative radiation therapy. Neck dissection was not performed in any of the cases. The size of the tumor ranged from 0.8 to 3.5 cm, with a mean of 1.8 cm. All cases were diagnosed as Stage I at the initial diagnosis. Although the follow-up period varied from 12 to 90 months, all patients were alive without disease after surgery.

Cytological Findings

The cytological features are summarized in Table 3. Cytological smears of all cases were cellular and exhibited many loosely cohesive syncytial clusters (Fig. 1). Many histiocytes were seen in the background or intermingled with tumor cells, and some of

them contained hemosiderin pigments (Fig. 2A). In 6 cases, variously shaped mucinous material was identified in the background or within the epithelial clusters (cases 1, 2, 3, 4, 5 and 7) (Fig. 2B). Many small lymphocytes derived from lymphoid infiltrate in the stroma of tumors were found among tumor cell clusters in cases 4, 5, 6 and 7.

In 2 cases that had predominant papillary-cystic components histologically, many true papillary clusters were evident (cases 3 and 5) (Fig. 3A). In addition papillary-looking clusters with capillaries derived from septa between cystic structures were occasionally seen (cases 1, 2 and 4) (Fig. 3B). Most cases showed small to medium-sized follicular structures with secreted materials (cases 1, 2, 3, 4, 5 and 7) (Fig. 4). Large flat epithelial sheets derived from cyst walls were also present in cases 1, 3, 4 and 5. The finding of many isolated cells was common, suggesting loose cohesion between the tumor cells. Necrotic cell clusters were rare and not inconspicuous (case 6).

Tumor cells appeared cuboidal, spindle to bipolar with elongated cytoplasmic tails or polygonal. Tumor cells exhibited small to medium-sized round to oval nuclei between 7.5 and 17.5 microns in diameter, with a smooth contour and indistinct or small nucleoli (Figs. 4, 5), except for case 4 that showed occasional enlarged distinct nucleoli. Tumor cells with vacuolated cytoplasm were common, and the size and shape of each vacuole varied considerably (Fig. 5). In case 1, occasional signet ring-like cells with mucin were present. No tumor cells displayed obvious acinic differentiation in the form of cytoplasmic zymogen granules.

Histopathological Findings

Histological patterns of the tumor are summarized in Table 3. Microcystic configurations were commonly seen in all cases, sometimes with other areas. Other architectural growth patterns included follicular in 4 cases (cases 1, 4, 5 and 7),

papillary-cystic in 2 (cases 3 and 5) (Fig. 6) and solid in one (case 6). In general, the tumor cell nuclei were bland, uniform, and round to oval, with a fine chromatin and inconspicuous nucleoli. In 4 of 7 cases, the tumor was composed of a mixture of eosinophilic cells and vacuolated cells (case 3, 4, 5 and 7), whereas vacuolated cells were a predominant component in the other cases (case 1, 2 and 6). Mitotic figures were scarcely identified.

Diastase-PAS and Alcian blue-positive mucinous materials were present within follicular or microcystic spaces (Fig. 7A). However, diastase-PAS-positive distinct zymogen granules were not detected in any cases.

Immunohistochemical findings are summarized in Table 4. Mammaglobin, S-100 protein, vimentin and MUC1 were consistently positive in the tumor cells (Fig. 7B-D). About mammaglobin, S-100 protein and vimentin, their staining patterns were usually strong and diffuse, but were focal in one case (case 6). Immunostaining for amylase was completely negative in all cases and p63 was focally positive in 2 cases. Ki-67 index ranged from 5.0 to 12.5%, with a mean of 7.8%.

Molecular Findings

RT-PCR analysis revealed that the *ETV6-NTRK3* fusion gene was identified in all 7 cases (Fig. 8). Furthermore, nucleotide sequencing of the RT-PCR fragment confirmed the presence of the identical fusion in all tested samples.

Discussion

Hirokawa et al. reported morphological similarity between secretory carcinoma of the breast and salivary gland acinic cell carcinoma in 2002.[17] In 2010, such a salivary gland tumor was first defined as a distinctive entity and designated “MASC” by Skálová et

al.[1]; it was stated characteristically to harbor a specific genetic alteration: t(12;15)(p13;q25), resulting in the formation of *ETV6-NTRK3* fusion gene, which is identical to that seen in secretory carcinoma of the breast. This is the third fusion gene detected in salivary gland malignancy after *MECT1-MAML2* in mucoepidermoid carcinoma and *MYB-NFIB* in adenoid cystic carcinoma.[18]

The clinicopathological features of our cases in terms of the mean age: 51.6 years, male-to-female ratio: 3:4 and site of tumors: the majority arising in the parotid gland among the major salivary glands, were comparable to previously reported data.[1-4,11] The prognosis of MASC is difficult to establish firmly at this stage. In the series by Skálová et al.,[4] 3 patients had recurrence and 2 of them died of metastatic disease or multiple local recurrence 2 and 6 years after initial treatment, respectively.[4] However, they were in an advanced stage (T3 and T4) at the diagnosis. In contrast, all patients in this study were at T1 and alive without recurrence, although the length of the follow-up period varied from 12 to 90 months.

Several studies on cytological features of MASC[10-14] described some common findings, which include variably cellular specimens, loosely cohesive epithelial clusters, low-grade bland-looking nuclei and somewhat vacuolated cytoplasm without distinct zymogen granules. Furthermore, some investigators reported that mucinous background was one of the cytological features characteristic of MASC. Our cases shared the common features mentioned above, and a hemosiderin-laden histiocyte-rich background was present in most of them. Additionally, in our cases, clusters of follicular and occasional papillary-looking clusters with capillaries derived from septa between cystic structures were evident together with true papillary clusters.

Before the diagnosis of MASC is made, acinic cell carcinoma should primarily be

excluded. Indeed, there are several common cytological features between classic acinic cell carcinoma and MASC: cellular specimens, loosely cohesive syncytial epithelial clusters and low-grade bland-looking nuclei.[19-21] However, classic acinic cell carcinoma has many intracytoplasmic metachromatic zymogen granules and shows granular to lacy cytoplasm homogeneously. On the other hand, many MASC cases characteristically show small follicular structures, highly vacuolated cytoplasm and background mucin or lots of histiocytes, suggesting secretory features but a lack of intracytoplasmic zymogen granules. Although the cytological features of MASC described above are not definitive and the final diagnosis is only made by the identification of *ETV6-NTRK3* fusion gene, these findings would be helpful for differential diagnosis of MASC. Distinction between MASC and zymogen granule-poor acinic cell carcinoma may be difficult by means of cytology alone.

MASC should also be cytologically distinguished from other benign and low-grade salivary gland tumors accompanied by mucinous background, which include pleomorphic adenoma, metaplastic Warthin tumor, low-grade mucoepidermoid carcinoma, adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma. Pleomorphic adenoma differs from MASC in the presence of distinct metachromatic stromal components intermingled with variously shaped myoepithelial cells. Warthin tumor with mucinous metaplasia exhibits a mixture of mucin-containing polygonal cells and oncocytes in the mucinous and necrotic background. In this tumor, however, highly vacuolated cells characteristic of MASC are absent. The feature of mucinous background with mucin-containing cells in MASC can be confused with low-grade mucoepidermoid carcinoma. MASC lacks clusters consisting of a mixture of epidermoid, mucin-containing, and intermediate cells that typically present in low-grade

mucoepidermoid carcinoma. Adenoid cystic carcinoma can be differentiated from MASC by the presence of very tight cohesive clusters composed of cells showing a high nuclear-cytoplasmic ratio, and distinct metachromatic hyaline balls. Since both MASC and polymorphous low-grade adenocarcinoma share similar cytological features in terms of the diversity of clustering patterns and the bland cytology, the differential diagnosis between the two neoplasms can be challenging. However, highly vacuolated tumor cells forming follicular structures with secreted materials characteristic of MASC are not a finding of polymorphous low-grade adenocarcinoma. In addition, polymorphous low-grade adenocarcinoma almost exclusively arises in the minor salivary glands.

The histopathological findings of our cases were consistent with those previously reported in the literature. In our cases, a predominant architectural feature of the tumor was a mixture of microcystic, follicular and papillary-cystic growth patterns, but a solid area was also seen in one case, where the transition between solid and microcystic components was found. Although solid proliferations have also been described in MASC,[1-3,10,14] they were a minority of the population compared with microcystic, follicular or papillary-cystic patterns, suggesting secretory features. Although a diastase-PAS- and Alcian blue-positive mucin-like material in the luminal spaces of the follicular and microcystic structures was evident in all cases, no diastase-PAS-positive intracytoplasmic zymogen granules were identified. Immunohistochemically, similar to other reports, S-100 protein, mammaglobin and vimentin were consistently positive in the tumor cells.

Although MASC has been reported histologically to mimic other low-grade salivary malignancies – low-grade cystadenocarcinoma, adenocarcinoma, not otherwise

specified, mucin-producing signet ring adenocarcinoma or mucoepidermoid carcinoma, most MASC cases have been categorized in the entity diagnosed previously as acinic cell carcinoma.[1-4] Acinic cell carcinoma has been recognized to show a wide range of cell types and architectural configurations with a feature of serous acinar differentiation, at least in some part of the tumor. The cell types present, other than serous acinar cells, include intercalated ductal, non-specific glandular, vacuolated and clear cells. Acinic cell carcinomas show a variety of growth patterns, which include solid, microcystic, papillary-cystic and follicular, and tumors may contain one or several of these architectural subtypes.[22] In acinic cell carcinomas, those with predominant vacuolated or clear cells and microcystic, papillary-cystic and follicular growth patterns without definite serous differentiation should be considered for the possibility of MASC. Because the fusion gene was not detectable in classic zymogen granule-rich acinic cell carcinoma,[4] so-called acinic cell carcinoma has been regarded as a heterogeneous group of entities.

Conclusions

MASC is a recently described salivary gland tumor that is defined by *ETV6-NTRK3* gene fusion. We reported the cytological as well as clinicohistological findings of 7 cases of genetically proven MASC cases. On fine-needle aspiration cytology, MASC should primarily be considered in the differential diagnosis of acinic cell carcinoma with secretory features. According to our cases, MASC was cytologically characterized by the presence of the clusters with follicular and occasional papillary structures composed of bland tumor cells exhibiting vacuolated cytoplasm, but lack of intracytoplasmic zymogen granules, in a mucinous or hemosiderin-laden histiocyte-rich

background.

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Figure Legends

Fig. 1.

A: Fine-needle aspiration cytology smear is highly cellular with many large clusters. B: Each cluster shows loosely cohesive and syncytial appearances (A, B: Case 2, Papanicolaou stain, A: x40, B: x400).

Fig. 2.

A: Clusters showing microfollicular structures and signet ring cell-like tumor cells containing mucin. Note that there are many histiocytes with coarse hemosiderin pigments in the background (Case 1). B: Irregularly shaped metachromatic mucin in the background (Case 7) (A: Papanicolaou stain, x400, B: May-Giemsa stain, x200).

Fig. 3.

A: Large clusters with papillary configurations (Case 3). B: Papillary-looking clusters with capillaries derived from follicular septa surrounded by many hemosiderin-laden macrophages (Case 1) (A, B: Papanicolaou stain, A: x100, B: x200).

Fig. 4.

A and B: Small follicular and loosely cohesive syncytial clusters with tumor cells exhibiting small bland-looking nuclei (Case 2) (A, B: Papanicolaou stain, x400).

Fig. 5.

Flat (A) and ball-like (B) clusters consisting of highly vacuolated tumor cells (A: Case 1, B: Case 3). Tumor cells exhibit small to medium-sized round to oval nuclei, with indistinct or small nucleoli. The size of vacuoles varies considerably: large in A and small in B (A, B: Papanicolaou stain, x400).

Fig. 6.

Histologically, the tumor shows microcystic (A), follicular (B) and papillary-cystic (C)

structures (A: Case 2, B: Case 1, C: Case 3). Note that luminal spaces are filled with secreted materials in the microcystic and follicular patterns (H and E stain, x200).

Fig. 7.

A: Microcystic structures contain diastase-PAS-positive mucinous material. Note no diastase-PAS-positive cytoplasmic granules in any of the tumor cells (diastase-PAS stain, x200). Immunohistochemically, tumor cells are diffusely positive for mammaglobin (B), S-100 protein (C) and vimentin (D) (B-D: immunostain, x200).

Fig. 8.

A: RT-PCR analysis shows amplification of *ETV6-NTRK3* fusion transcripts (1-7, case no.). M, molecular weight marker; NK, negative amplification control; PK, positive amplification control. B: Direct sequencing of amplified RT-PCR product confirms the presence of *ETV6-NTRK3* rearrangement.

Table 1.
Antibodies Used for Immunohistochemical Study

Antigen	Clone	Dilution	Source
Mammaglobin	304-1A5	Ready-to-use	DAKO, Glostrup, Denmark
S-100 protein	S100	1:2000	DAKO, Glostrup, Denmark
Vimentin	V9	1:200	DAKO, Glostrup, Denmark
MUC-1	Ma695	1:200	Novocastra Laboratories Ltd, Newcastle, UK
p63	4A4	1:200	DAKO, Glostrup, Denmark
Ki-67	MIB1	1:2000	DAKO, Glostrup, Denmark

Table 2.
Clinicopathological Features

Case	Age	Gender	Location	Tumor size (cm)	pStage	Treatment	Follow up
1	51	Female	Right submandibular	3.5 x 3.0	T1N0M0	OP	NED, 79 mo
2	39	Male	Left parotid	1.5 x 1.5	T1N0M0	OP	NED, 48 mo
3	68	Female	Right parotid	0.8 x 0.7	T1N0M0	OP +RT	NED, 12 mo
4	47	Male	Accessory parotid	1.3 x 0.8	T1N0M0	OP	NED, 25 mo
5	46	Female	Right parotid	2.0 x 1.5	T1N0M0	OP	NED, 10 mo
6	56	Female	Left parotid	1.7 x 1.3	T1N0M0	OP	NED, 90 mo
7	54	Male	Right parotid	1.5 x1 .5	T1N0M0	OP	NED, 12 mo

OP: operation, RT: radiation therapy, NED: no evidence of disease.

Table 3.
Histological and Cytological Findings

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Histologic Pattern	Follicular/ Microcystic	Microcystic	Papillary-cystic/ Microcystic	Follicular/ Microcystic	Papillary-cystic/ Microcystic/ Follicular	Solid/ Microcystic	Microcystic/ Follicular
Cytologic cellularity	High	High	High	High	High	High	High
Cellular arrangement	Loosely cohesive, syncytial, papillary looking clusters with capillaries, flat sheets, small follicular, and isolated cells	Loosely cohesive, syncytial, papillary looking clusters with capillaries,,small follicular, and isolated cells	Loosely cohesive, syncytial, papillary, flat sheets, ball-like, and small follicular	Loosely cohesive, syncytial, papillary looking clusters with capillaries, flat sheets, small follicular, and isolated cells	Loosely cohesive, syncytial, papillary, flat sheets, small to medium-sized follicular, and isolated cells	Loosely cohesive, syncytial, isolated cells, necrosis, and transgressing capillaries	Loosely cohesive, syncytial, small follicular, isolated cells, and transgressing capillaries
Cytoplasmic features	Small to medium; Polygonal, cuboidal, and bipolar; Variously sized vacuoles, occasional signet ring cells	Small; Cuboidal, bipolar, and polygonal; Various-sized vacuoles and lacy	Small to medium; Cuboidal, and polygonal; Various-sized vacuoles	Small to large; Polygonal, bipolar, and cuboidal; Granular to lacy, occasional various-sized vacuoles	Small; Cuboidal, polygonal, and bipolar; Granular, small vacuoles, and occasional medium- to large-sized vacuoles	Small to medium; Cuboidal, spindle, and polygonal; Lacy and various-sized vacuoles	Small to medium; Polygonal; Lacy and various-sized vacuoles

Zymogen granules	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct
Nuclei	Round to oval; Smooth contour; Fine chromatin	Round to oval; Smooth contour; Fine chromatin	Round to oval; Smooth contour; Fine chromatin	Round to oval; Smooth contour; Fine chromatin	Round to oval; Smooth contour; Fine chromatin	Round to oval; Smooth contour; Fine chromatin	Round to oval; Smooth contour; Fine chromatin
Nucleoli	Indistinct or small	Indistinct or small	Indistinct	Occasionally distinct and enlarged	Indistinct or small	Indistinct or small	Indistinct or small
Background	Histiocytes with hemosiderin and mucin	Histiocytes with hemosiderin and mucin	Histiocytes with hemosiderin and mucin	Histiocytes with hemosiderin, lymphocytes, and mucin	Histiocytes with hemosiderin, lymphocytes, and mucin	Histiocytes with hemosiderin and lymphocytes	Histiocytes with hemosiderin, lymphocytes, and mucin

Table 4.
Immunohistochemical Features

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Mammaglobin	+	+	+	+	+	+, focal	+
S-100 protein	+	+	+	+	+	+, focal	+
Vimentin	+	+	+	+	+	+	+
MUC-1	+, focal	+	+	+	+	+	+
Amylase	-	-	-	-	-	-	-
p63	-	-	-	-	-	+, focal	+, focal
Ki-67 index (%)	5.0	6.0	6.5	7.8	8.5	12.5	8.1

Fig.1

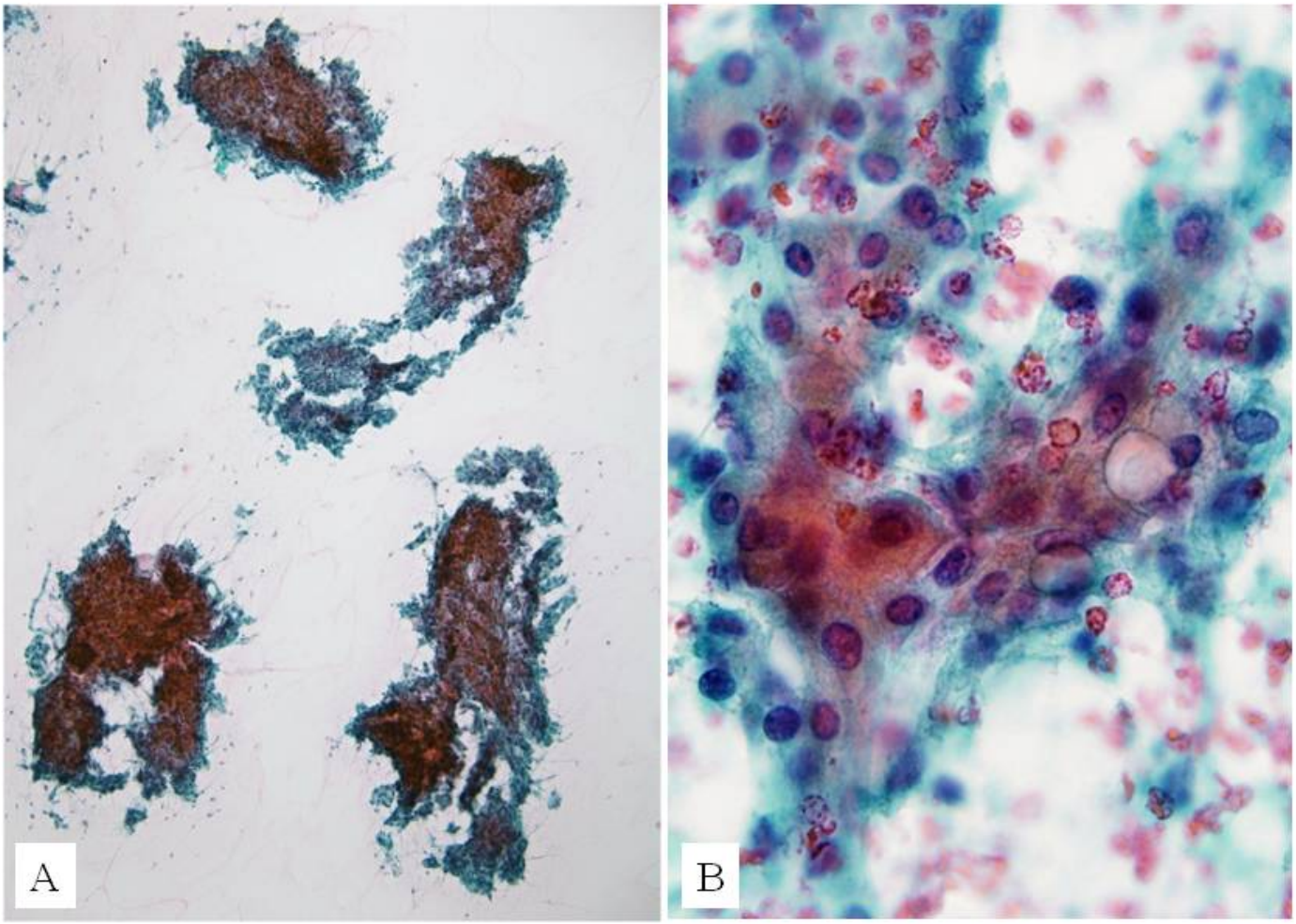


Fig.2

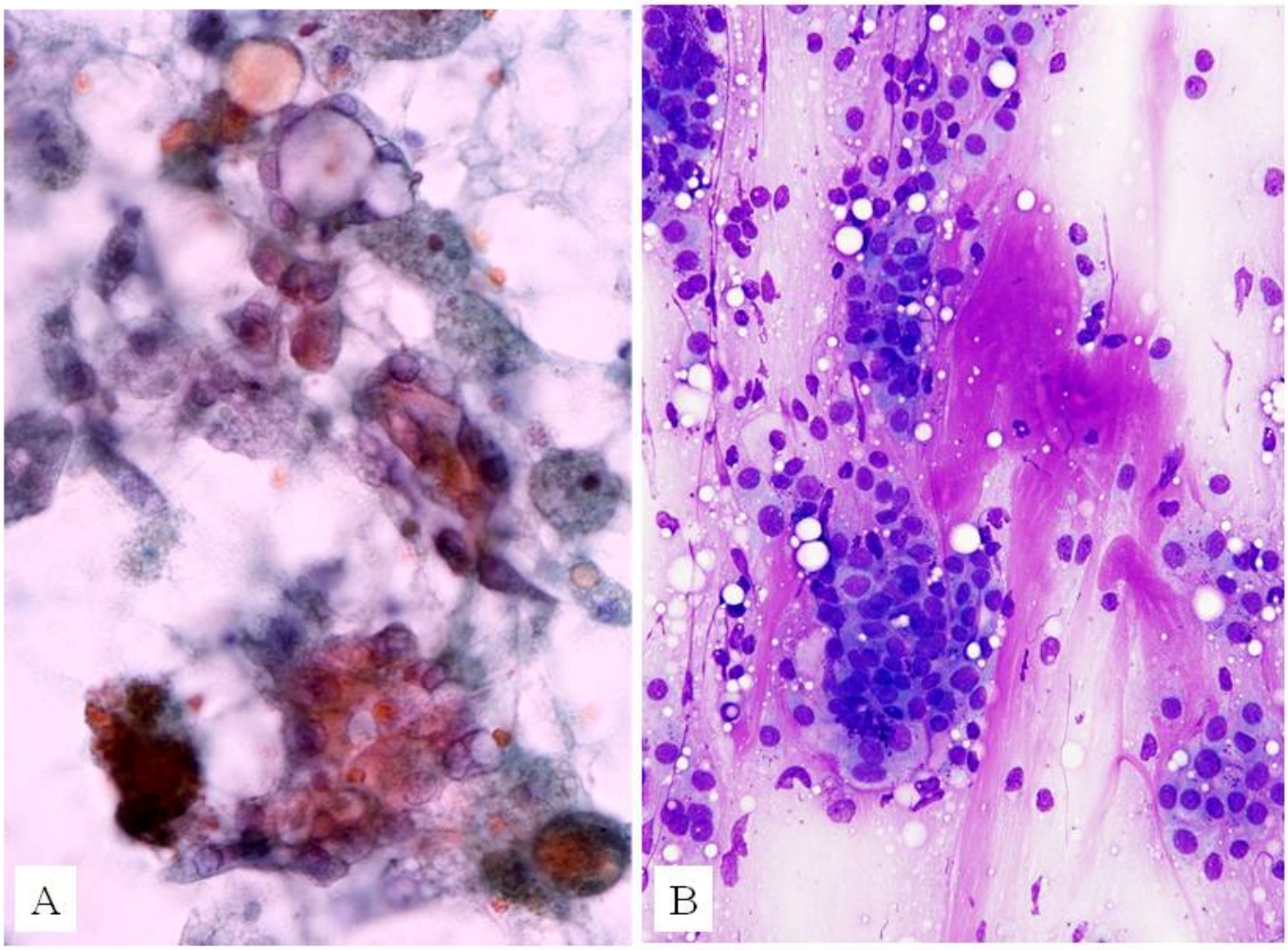


Fig.3

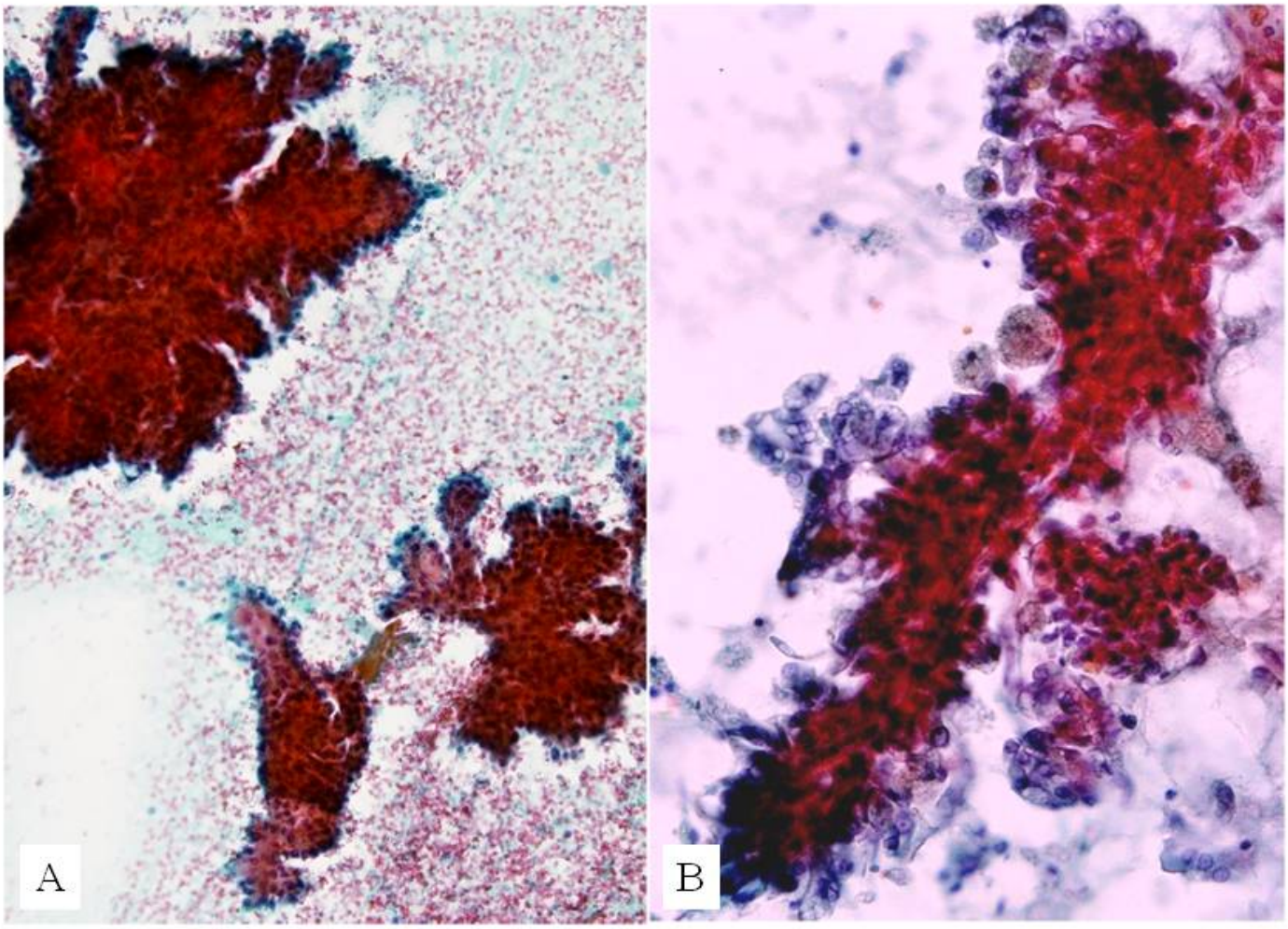


Fig.4

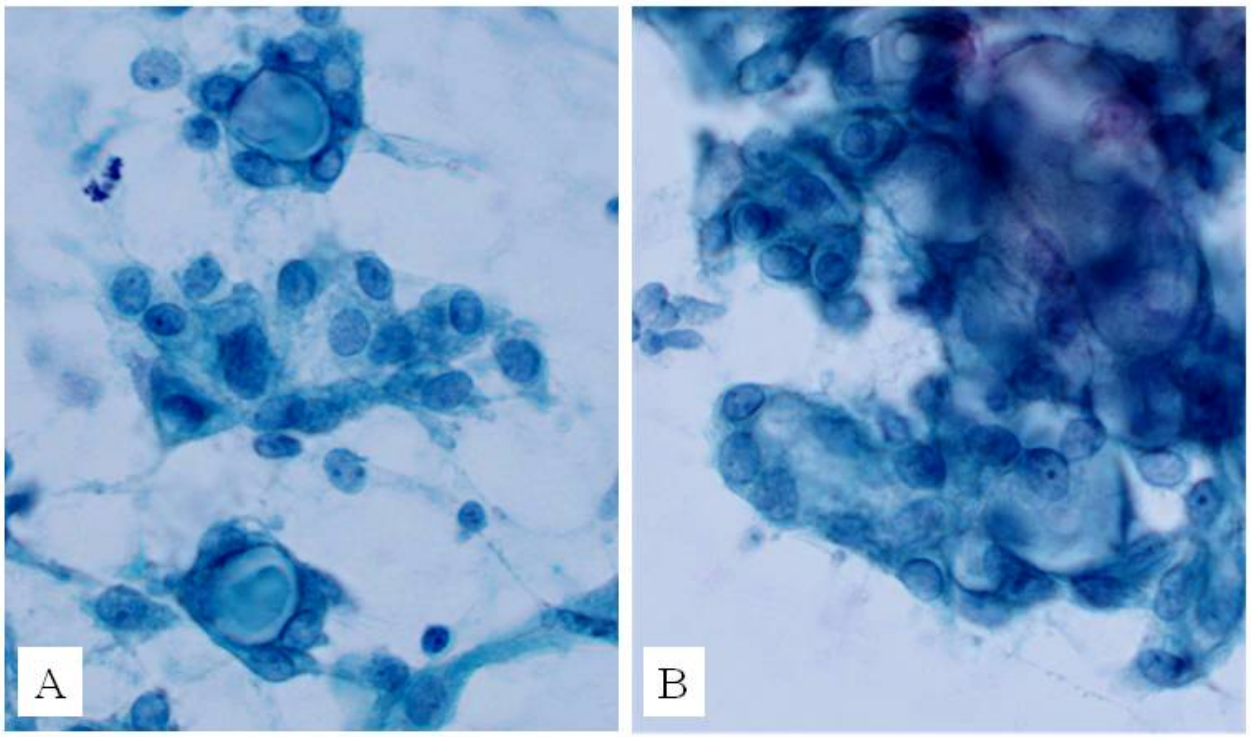


Fig.5

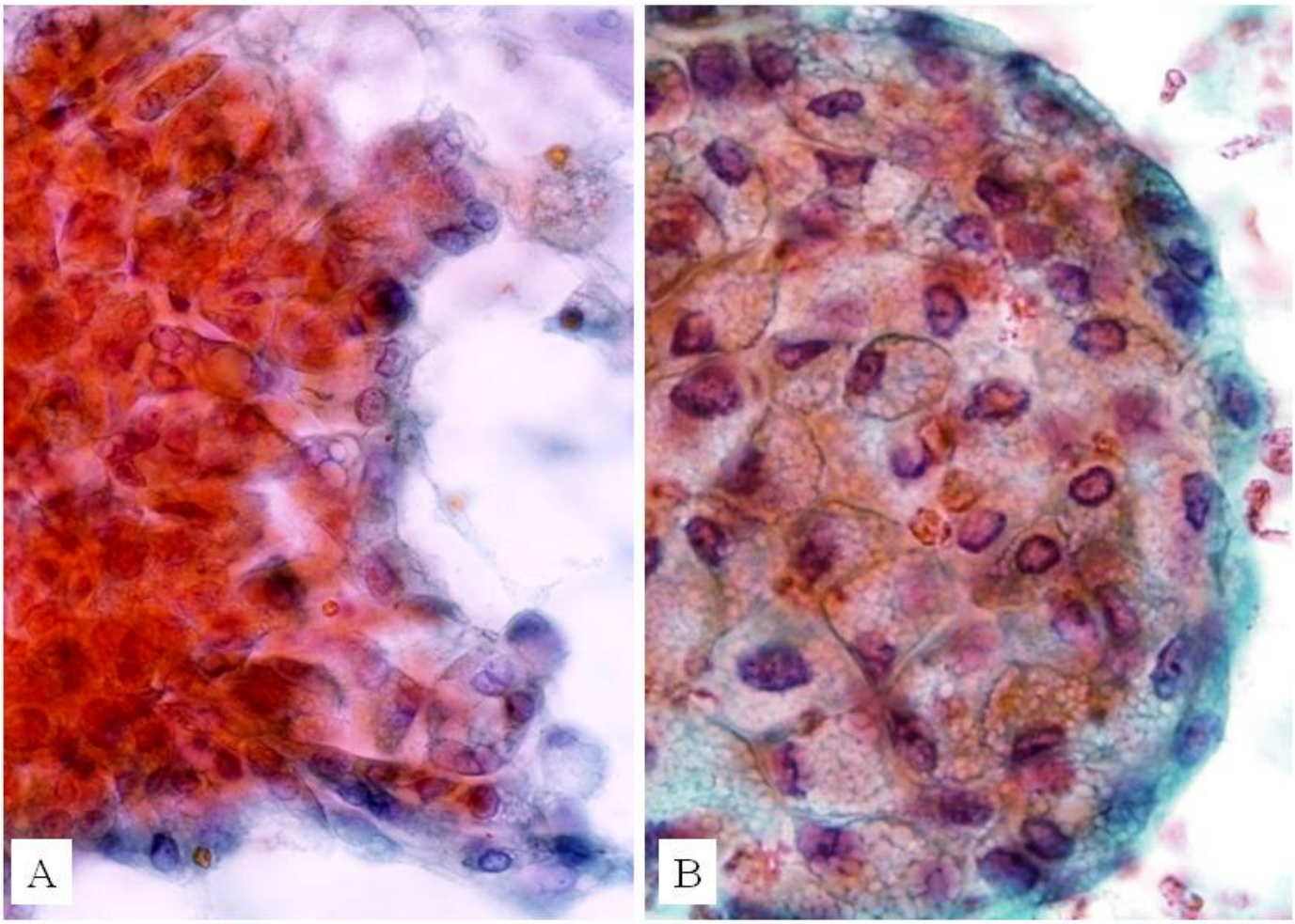


Fig.6

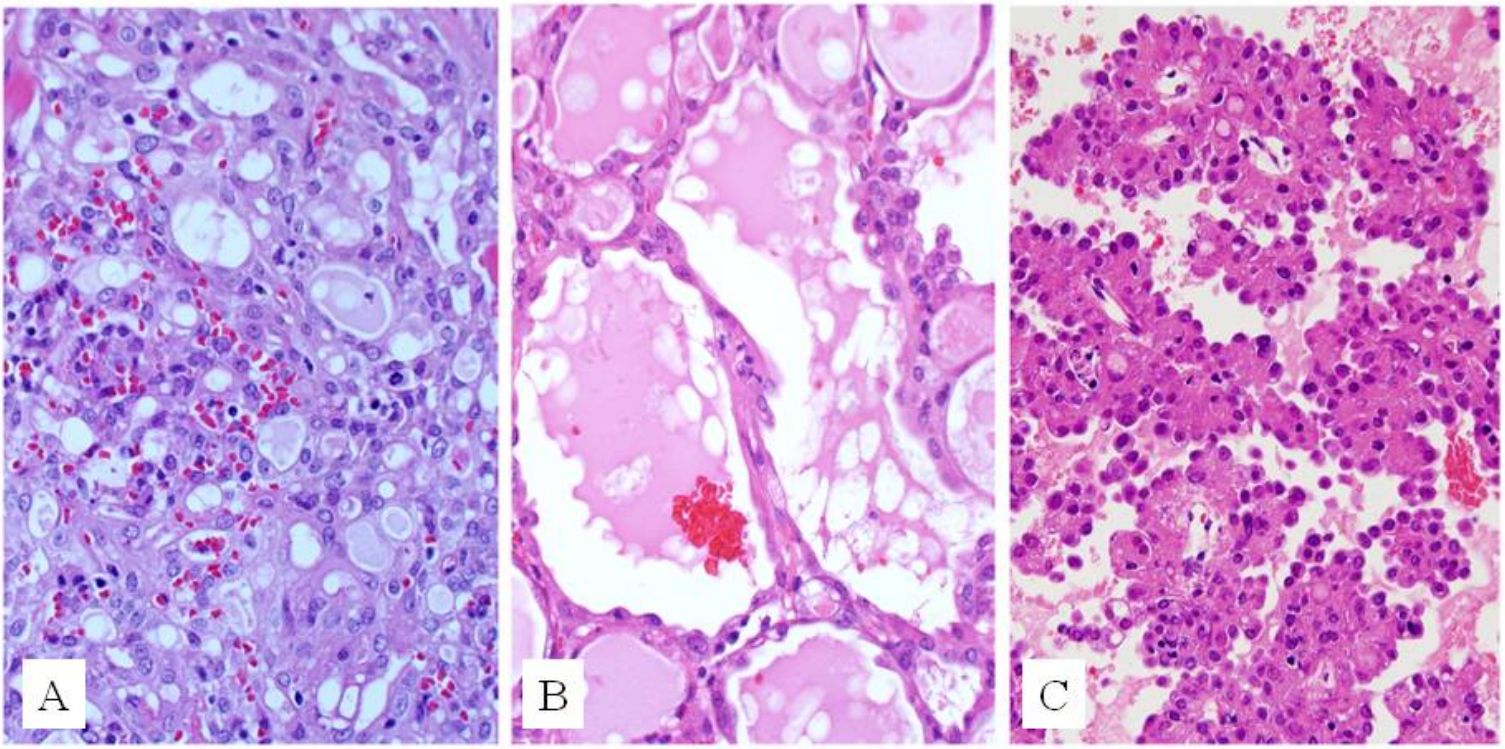


Fig.7

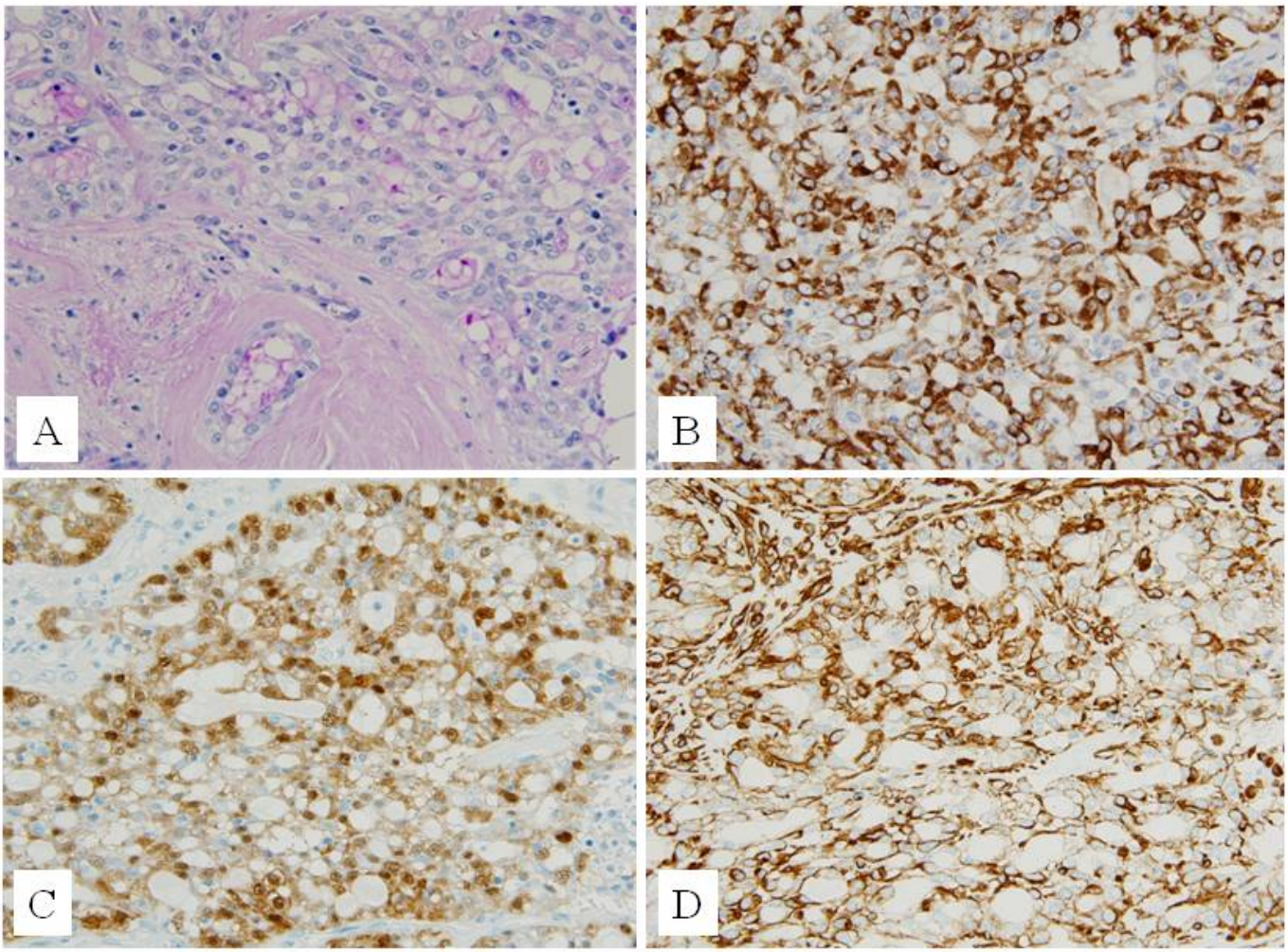
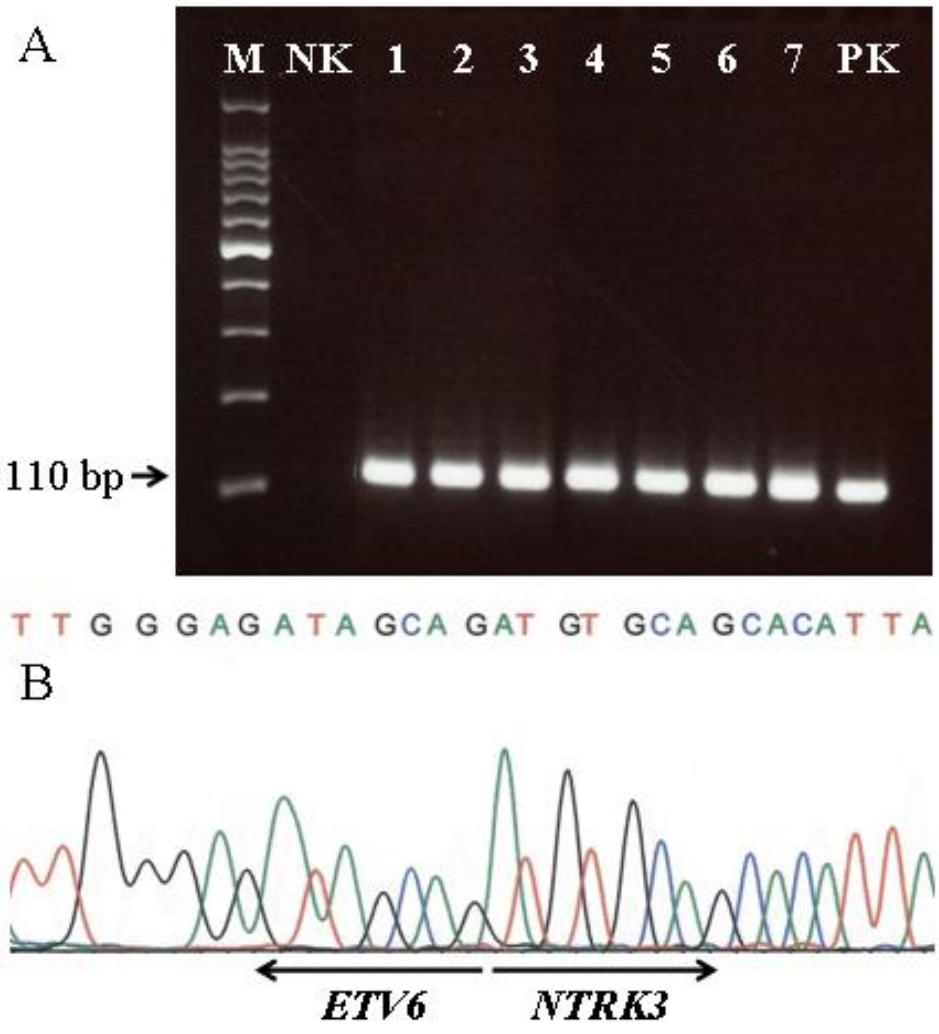


Fig.8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T T G G G A G A T A G C A G A T G T G C A G C A C A T T A
B
ETV6
NTRK3