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Novel Insights from Clinical Practice

Title:

Cytologic features of SMARCA4-deficient thoracic sarcoma: a case report and comparison with other SWI/SNF complex-deficient tumors

Running title:

Cytology of SMARCA4-DTS

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Key words:

SMARCA4-deficient thoracic sarcoma; Rhabdoid cells; SWI/SNF chromatin remodeling complexes; Immunocytochemistry

Established facts:

- SMARCA4-deficient thoracic sarcoma is a recently proposed entity whose cytologic features have not yet been well-described in the literature.
- SWI/SNF chromatin remodeling complexes are known to be involved in the pathogenesis of various human malignancies, some of which are characterized by rhabdoid cells.

Novel insights:

- Cytology of SMARCA4-DTS is characterized by high cellularity, atypical round-to-polygonal cells that appear singly or in loose clusters, enlarged nuclei with vesicular nuclei, prominent nucleoli, and some cells showing rhabdoid morphology.
- The above cytologic features are not unique to SMARCA4-DTS, but sufficiently characteristic to suspect this tumor in the context of supporting clinical and radiological features.
- SMARCA4-DTS shares many cytologic features with several other SWI/SNF complex-deficient tumors, suggesting a causal link between SWI/SNF complex deficiencies and specific cytomorphologic changes.

Abstract

Background: SMARCA4-deficient thoracic sarcoma is a recently proposed entity of soft tissue tumors associated with an extremely poor prognosis. Its cytologic features have not been well-described in the literature yet.

Case: A woman in her early 30s who presented with chest pain was found to have a tumor in the right chest wall. Cytologic smears revealed numerous atypical round-to-polygonal cells appearing singly or in loosely cohesive clusters. These cells had a well-defined cell border, scant to moderate cytoplasm, and enlarged vesicular nuclei with prominent nucleoli. In addition, some cells with eosinophilic globular intracytoplasmic inclusions and eccentrically located nuclei, consistent with rhabdoid cells, were observed. Immunocytochemically, the cells were at least focally positive for cytokeratin CAM5.2 and CD34 and showed a significantly reduced BRG1/SMARCA4 expression. The diagnosis was confirmed by histological, immunohistochemical, and genetic analysis of a metastatic lesion to the left axillary lymph node.

Conclusion: Although the cytologic features of SMARCA4-deficient thoracic sarcoma are not fully unique, they are sufficiently characteristic to suspect this tumor in cases of supporting clinical and radiological features, which may promote additional immunological or molecular testing to establish a definitive diagnosis.

Introduction

Recently, a distinct subset of intrathoracic undifferentiated tumors termed SMARCA4-deficient thoracic sarcomas (SMARCA4-DTSs) were described by Le Loarer et al. [1]. These tumors are characterized by the inactivation of *SMARCA4*, a gene encoding an ATPase subunit of the Switch/Sucrose Non-Fermenting (SWI/SNF) chromatin remodeling complexes. They have been shown to have unique clinicopathological features, exhibiting exclusive occurrence in the intrathoracic region, adult onset, frequent association with a history of smoking, undifferentiated round-to-polygonal epithelioid cell histology, and extremely poor prognosis with limited response to chemotherapies. Gene expression analysis revealed that these tumors are transcriptionally related to other SWI/SNF complex-deficient tumors—such as malignant rhabdoid tumors (MRTs) and small cell carcinomas of the ovary, hypercalcemic type (SCCOHTs)—but distinct from lung cancers with or without *SMARCA4* mutations [1]. Subsequent studies [2,3] also identified tumors corresponding to this new category, supporting the distinctiveness of SMARCA4-DTS from other potentially related entities.

We recently reported a case of SMARCA4-DTS and described how to diagnose this tumor using limited samples and resources [4]. During the course of the disease, the patient underwent cytologic examinations several times for the evaluation of

metastatic disease. Here, we report the features of these specimens. To the best of our knowledge, this report is the first cytologic description of this new entity.

Case Report

A female patient in her early 30s presented with right-sided chest pain and was found to have a tumor at the right chest wall. The full clinicopathological and molecular features of the tumor were described previously [4]. Briefly, biopsy of the tumor revealed sheets of undifferentiated round-to-polygonal cells, with a few cells showing rhabdoid morphology. Immunohistochemically, the tumor was positive for cytokeratin CAM5.2, vimentin, and CD34 and showed reduced BRG1/SMARCA4 expression with a complete loss of BRM/SMARCA2, suggesting a diagnosis of SMARCA4-DTS. The patient was initially treated with doxorubicin monotherapy; however, the tumor rapidly enlarged. Five months after her initial visit, the patient developed lymph node swelling in the left axillary region, for which fine needle aspiration cytology and a subsequent lymphadenectomy were performed. Imprint smears were made from the resected specimen. Shortly thereafter, the patient developed multiple metastases with ascites, and underwent abdominal paracentesis before receiving a chemotherapy-based treatment. A sample of the ascitic fluid was submitted for cytologic examination.

For each specimen, 95% alcohol-fixed smears and air-dried smears were prepared and stained with Papanicolaou stain and May-Grünwald-Giemsa (MGG) stain. Immunocytochemistry for cytokeratin CAM5.2, CD34, and BRG1/SMARCA4 was also performed using alcohol-fixed smears. Briefly, after blocking endogenous peroxidase activity using 3% hydrogen peroxide, slides were incubated with primary antibodies for 60 min. Then the slides were washed twice in phosphate buffered saline and incubated with a secondary antibody (Histofine® Simple Stain MAX PO, Nichirei, Tokyo, Japan) for 30 min. After washing twice, the slides were stained with diaminobenzidine and counterstained with hematoxylin. All operations were carried out at room temperature. Clones and staining conditions of primary antibodies were as shown in Table 1. Since the resected lymph node was available for histological and molecular analysis to establish the diagnosis, the cell block technique was not applied to any of these cytologic samples.

The cytologic findings were similar in the three specimens. The smears were moderately to highly cellular and contained numerous atypical round-to-polygonal cells that appeared singly or in loosely-cohesive clusters in a necrotic background (Figure 1A). The cells had a well-defined cell border, scant to moderate cytoplasm, enlarged nuclei with vesicular chromatin, and prominent nucleoli. Some cells with irregularly invaginated nuclear membrane and perinuclear cytoplasmic densities were noted

(Figure 1A, inset). In addition, some cells with eosinophilic globular intracytoplasmic inclusions and eccentrically located nuclei, consistent with rhabdoid morphology, were observed (Figure 1B). Mitotic figures were frequently seen. In the MGG-stained smears, numerous small vesicles were noted in the cytoplasm (Figure 1C).

Immunocytochemically, the cells were at least focally positive for CAM5.2 and CD34 and showed significantly reduced expression of BRG1/SMARCA4 (Figure 1D–F), similar to the results for tissue sections [4]. Considering the patient's clinical course, a cytologic diagnosis of metastatic SMARCA4-DTS was made in all the three examinations.

Histology of the excised lymph node showed a tumor composed of sheets of atypical round-to-polygonal cells, which was identical to the primary tumor (Figure 2A). Immunohistochemically, it showed reduced BRG1/SMARCA4 expression and complete loss of BRM/SMARCA2 expression (Figure 2B–C). The expression of INI1/SMARCB1 was retained. Moreover, the tumor was completely negative for claudin-4, indicating a lack of epithelial differentiation (Figure 2D). Sanger sequencing using DNA extracted from the lymph node revealed a nonsense c.1546A>T (p.516Lys>Ter) mutation in *SMARCA4* [4]. Collectively, the diagnosis of metastatic SMARCA4-DTS was established.

Discussion

The cytologic features described here recapitulate the histology of SMARCA4-DTSs [2,3]. These tumors have been shown to be histologically similar among cases, displaying diffuse sheets of poorly cohesive, relatively monotonous undifferentiated ovoid/epithelioid cells with vesicular chromatin, and prominent nucleoli. Varying proportions of rhabdoid cells were found in 50% to 100% of cases, and brisk mitotic activity and necrosis were noted as universal features. Moreover, immunocytochemistry demonstrated that cytologic specimens can be used to replicate the characteristic immunophenotype of this tumor. Although SMARCA4-DTS seems to be quite rare, many patients with this tumor present at an advanced stage and are likely to undergo cytologic examination; thus, cytopathologists should be fully aware of the cytologic features of this tumor. We believe that our case will serve as a good reference for future cases of suspected SMARCA4-DTS.

The cytologic differential diagnosis of SMARCA4-DTS includes various high-grade malignancies such as poorly-differentiated carcinomas, mesothelioma, melanoma, rhabdomyosarcoma, epithelioid angiosarcoma, and some kinds of lymphomas. What is important here is rhabdoid morphology is not specific to SWI/SNF complex-deficient tumors, since it may be encountered in a variety of other neoplasms [5]. Therefore, the

diagnosis of SMARCA4-DTS cannot be established by morphology alone. Therefore, immunocytochemistry or immunohistochemistry using cell blocks from cytologic specimens may particularly be useful, since this tumor characteristically co-expresses CD34, vimentin, and epithelial markers (cytokeratins or epithelial membrane antigen) in conjunction with the complete absence or diffuse reduction of BRG1/SMARCA4 expression and loss of BRM/SMARCA2 expression [1–4]. However, since some cytokeratin cocktails such as AE1/AE3 may stain rare cells [1–3], the use of multiple antibodies is recommended. For example, CAM5.2 staining was more robust in our case, suggesting the superiority of this antibody in this setting.

Currently, the distinction between SMARCA4-DTS and poorly differentiated lung carcinomas is not entirely clear because (i) loss of SMARCA4/BRG1 may also be observed in a subset of lung carcinomas, (ii) patients with SMARCA4-DTS mostly present at an advanced stage and frequently have pulmonary involvement, and (iii) many SMARCA4-DTSs are diagnosed by biopsy, which does not exclude the possibility of more differentiated components in the tumors. However, recent studies have suggested that claudin-4 is a useful marker for clearly distinguishing between these two entities. Schaefer et al. performed immunohistochemistry for claudin-4 on various tumors and found that the expression of claudin-4 was frequent in carcinomas (including SWI/SNF complex-deficient ones) and epithelial components of synovial

sarcomas, whereas almost all the sarcomas investigated were negative for this marker [6]. Indeed, a uniform lack of claudin-4 expression in SMARCA4-DTS was observed in a case series by Yoshida et al. [2] as well as in our case, suggesting that SMARCA4-DTSs are true sarcomas and do not represent the least differentiated variants of lung carcinomas.

Differences in CD34 expression may also distinguish SMARCA4-DTS from lung carcinomas: CD34 is expressed in the majority (60–83%) of SMARCA4-DTSs [1–3], whereas this marker is not usually expressed in lung carcinomas including SMARCA4-deficient ones [2]. However, in endometrial carcinomas, Shah et al. recently reported that CD34 was not uncommonly expressed in undifferentiated components of dedifferentiated carcinomas, suggesting that CD34 cannot be used to exclude epithelial neoplasms [7]. Therefore, the distinction between SMARCA4-DTS and poorly differentiated lung carcinomas cannot be made solely based on CD34 expression; rather, it should be done in conjunction with careful assessment of other clinicopathological and immunohistochemical findings.

In this case study, we also compared cytologic features of the present tumor with those of other SWI/SNF complex-deficient tumors described in the literature. This revealed that SMARCA4-DTS shares many cytomorphologic features with proximal-type

epithelioid sarcomas [8–11], MRT [12–15], and SCCOHT [16–20]. In the latter tumors, smears are generally highly cellular and composed of numerous round-to-polygonal cells that appear as single cells or in loose clusters without structures. These cells have enlarged atypical nuclei with vesicular chromatin and nucleoli are usually prominent. The amount of cytoplasm is reported to vary, but is always abundant in proximal-type epithelioid sarcomas but less abundant or scant in MRTs and SCCOHTs. Moreover, rhabdoid cells or cells with perinuclear hyaline inclusions have been frequently noted in these three neoplasms [8,9,11,12,14–16,18,20]. Perinuclear cytoplasmic densities (denser in the inner area than in the periphery) have also been noted [9,15]. Consequently, it can be concluded that SWI/SNF complex deficiency results in the common cytomorphologic changes into undifferentiated epithelioid cells with decreased cohesiveness, with occasional cells showing rhabdoid features. Indeed, a French group recently pointed out an important morphological similarity between MRT and SCCOHT by directly comparing cases [20]. Therefore, it is highly unlikely that these tumors can be accurately differentiated solely on the basis of cytomorphologic analysis. Adequate clinicoradiological information, such as patient age, tumor location, smoking history, and familial history, as well as sufficient immunophenotypic analysis is mandatory for establishing the correct diagnosis. Since MRT occurs almost exclusively in infants and young children, it can be readily differentiated from SMARCA4-DTS. On the other hand, proximal-type epithelioid sarcoma occurs at any age and can arise in

the chest wall [21] and, thus, cannot be differentiated from SMARCA4-DTS by clinical presentation. However, larger tumor cells with abundant cytoplasm and loss of INI1/SMARCB1 expression may support the diagnosis of proximal-type epithelioid sarcoma rather than SMARCA4-DTS.

In summary, we described the cytologic features of SMARCA4-DTS arising in an adult female and discussed the differential diagnosis of this newly-described entity. Further studies using more cases are needed, because patients with this tumor are expected to frequently undergo cytologic examinations, and cytopathology specimens are the diagnostic materials available for many of these patients.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Conflict of Interest:

There is no potential conflict of interest associated with this manuscript.

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Author Contributions

M.M. performed immunocytochemistry and immunohistochemistry. S.K. performed genetic analysis. Both M.M. and S.K. assessed findings of the cytologic specimens and wrote the manuscript.

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Figure legends:

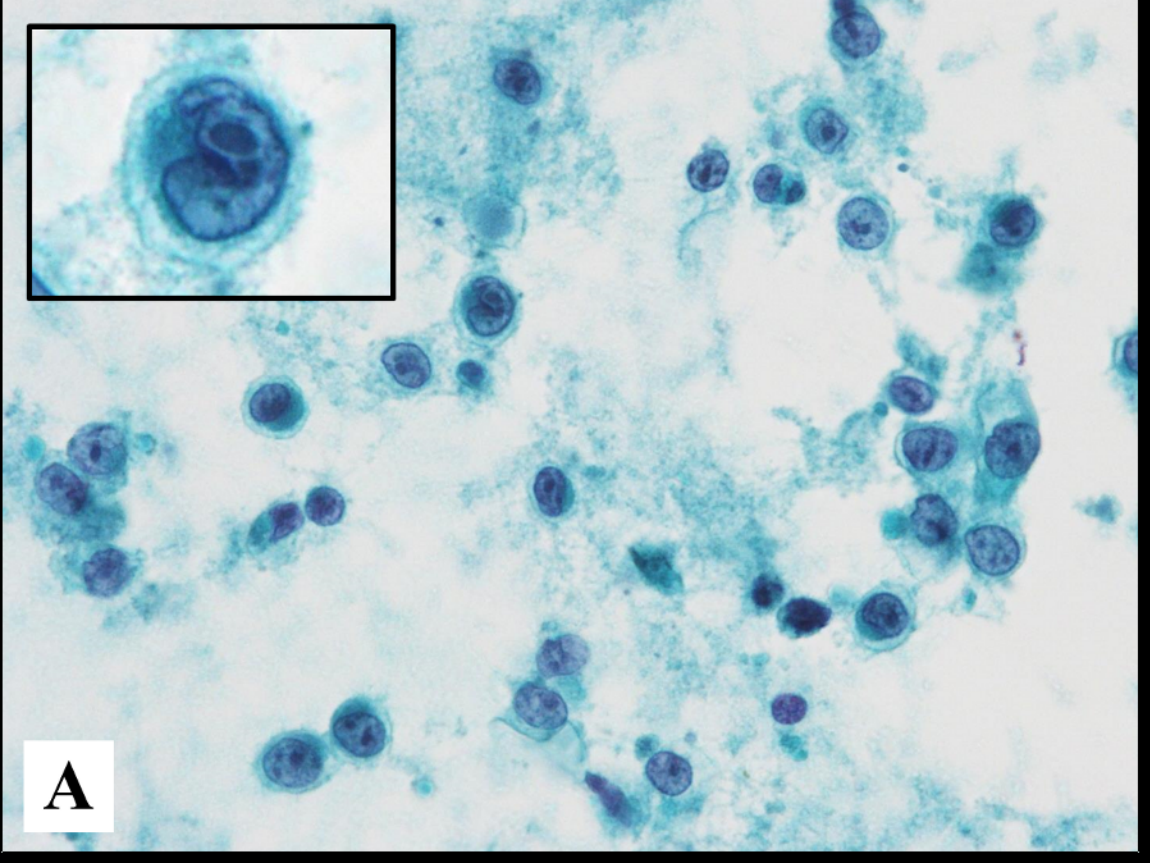
Figure 1

Cytology of SMARCA4-deficient thoracic sarcoma. (A) Imprint smear of the lymph node showing atypical round-to-polygonal cells that appear as single cells or in loosely-cohesive clusters in a necrotic background (Papanicolaou stain, x400). Some cells show irregularly invaginated nuclear membrane and perinuclear cytoplasmic densities (inset). (B) Ascitic fluid cytology showing large atypical cells with moderate amount of cytoplasm, enlarged nuclei, vesicular chromatin, and prominent nucleoli. Some cells have eosinophilic globular intracytoplasmic inclusions with eccentrically located nuclei, consistent with rhabdoid morphology (Papanicolaou stain, x1000). (C) Acitic fluid cytology. Numerous small vesicles were observed in the cytoplasm of atypical cells. (May-Grünwald-Giemsa stain, x1000). (D–F) Immunocytochemistry showing expression of cytokeratin CAM5.2 (D) and CD34 (E) and a significantly reduced expression of SMARCA4 (F) in the atypical cells (x1000). Note nonneoplastic cells in the background showing a strong immunoreactivity for SMARCA4 and serving as internal positive controls (arrows).

Figure 2

Histology and immunohistochemistry of the lymph node metastasis of SMARCA4-deficient thoracic sarcoma. (A) The tumor is composed of sheets of atypical

round-to-polygonal cells showing enlarged nuclei with vacuolar chromatin and prominent nucleoli. Rhabdoid cells are not apparent in this field (Hematoxylin-eosin stain, x600). (B,C) The tumor cells show a reduced BRG1/SMARCA4 expression (B) and a complete loss of BRM/SMARCA2 expression (C). Note nuclear staining in endothelial cells serving as internal positive controls (x600). (D) The tumor cells are completely negative for claudin-4 (x600). An external control tissue (normal tonsillar epithelium) is stained correctly (inset).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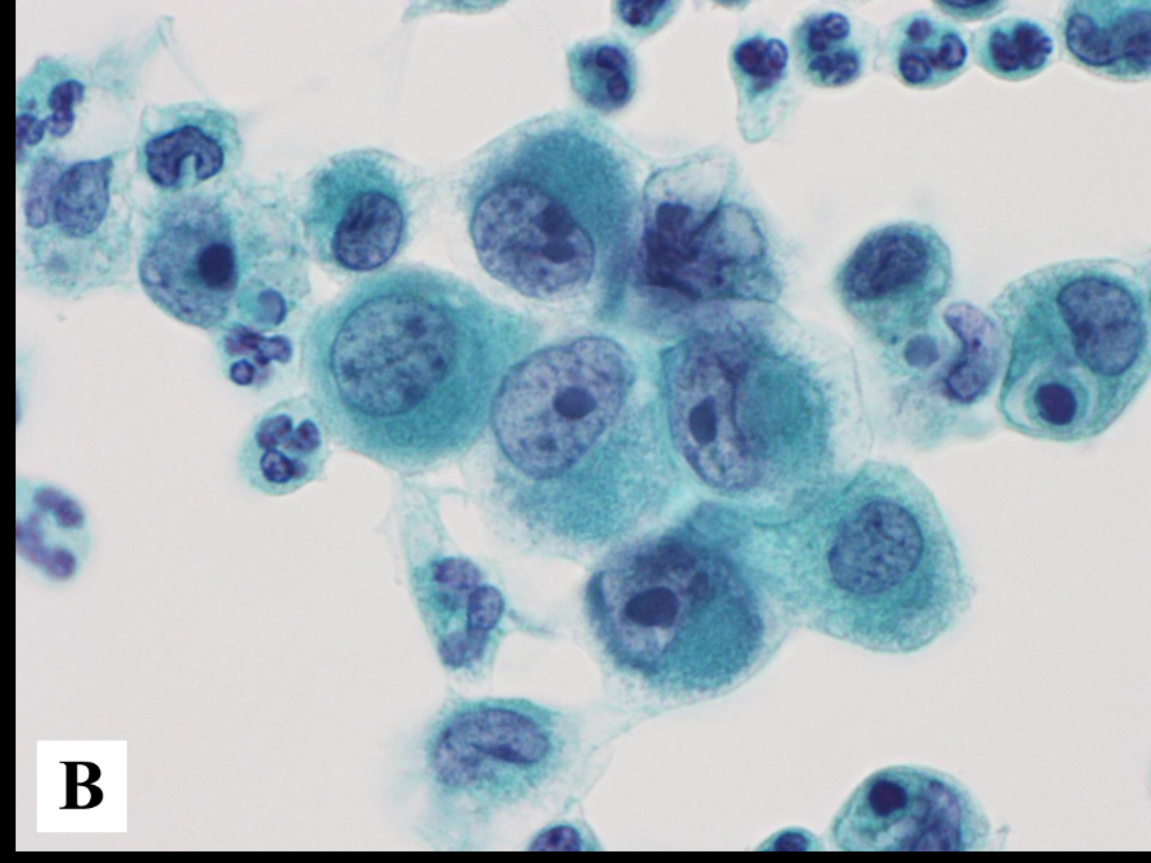
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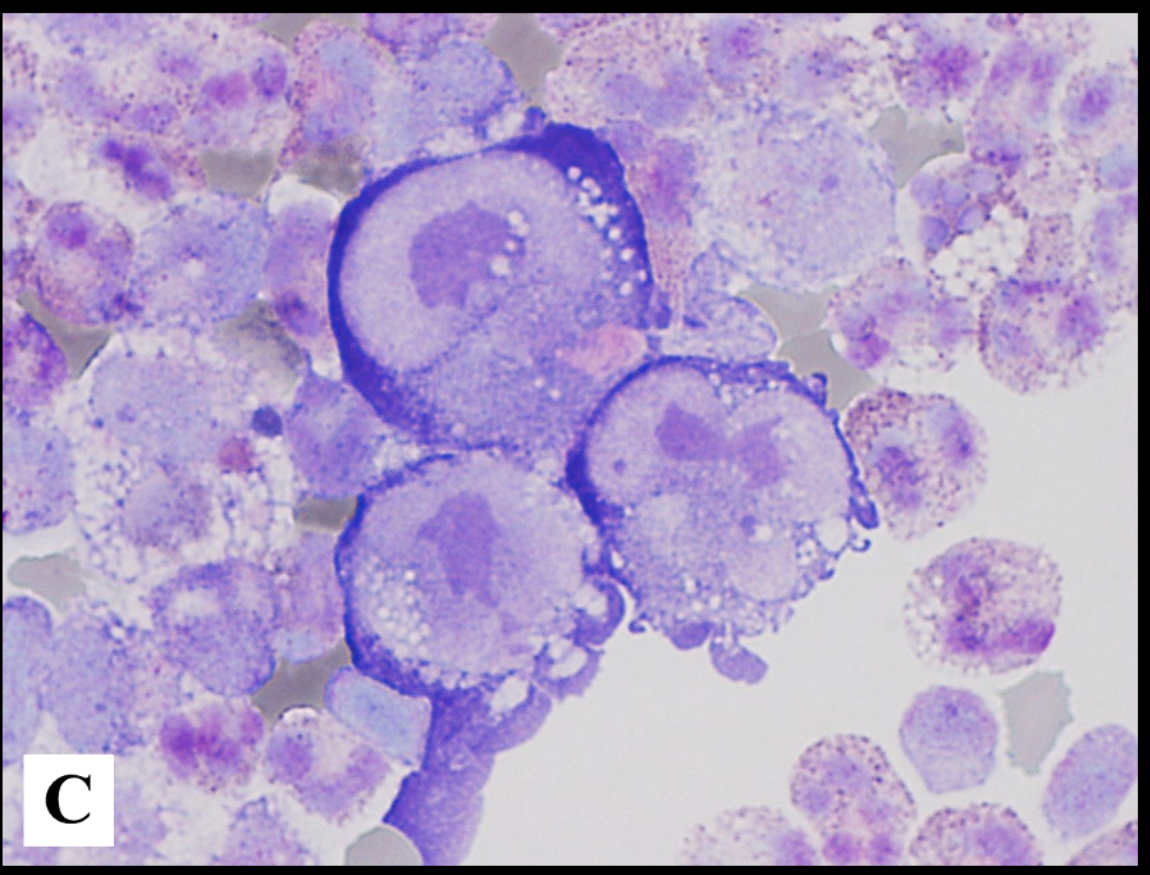
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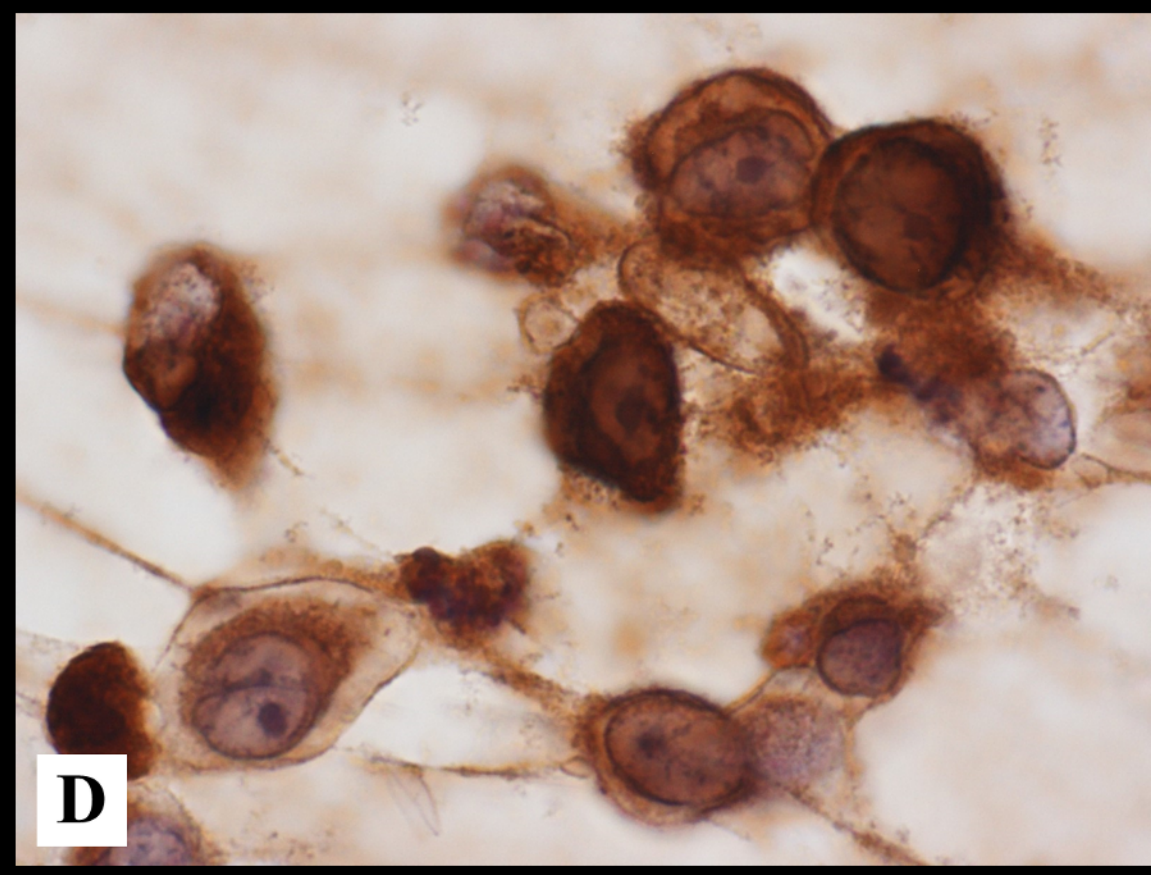
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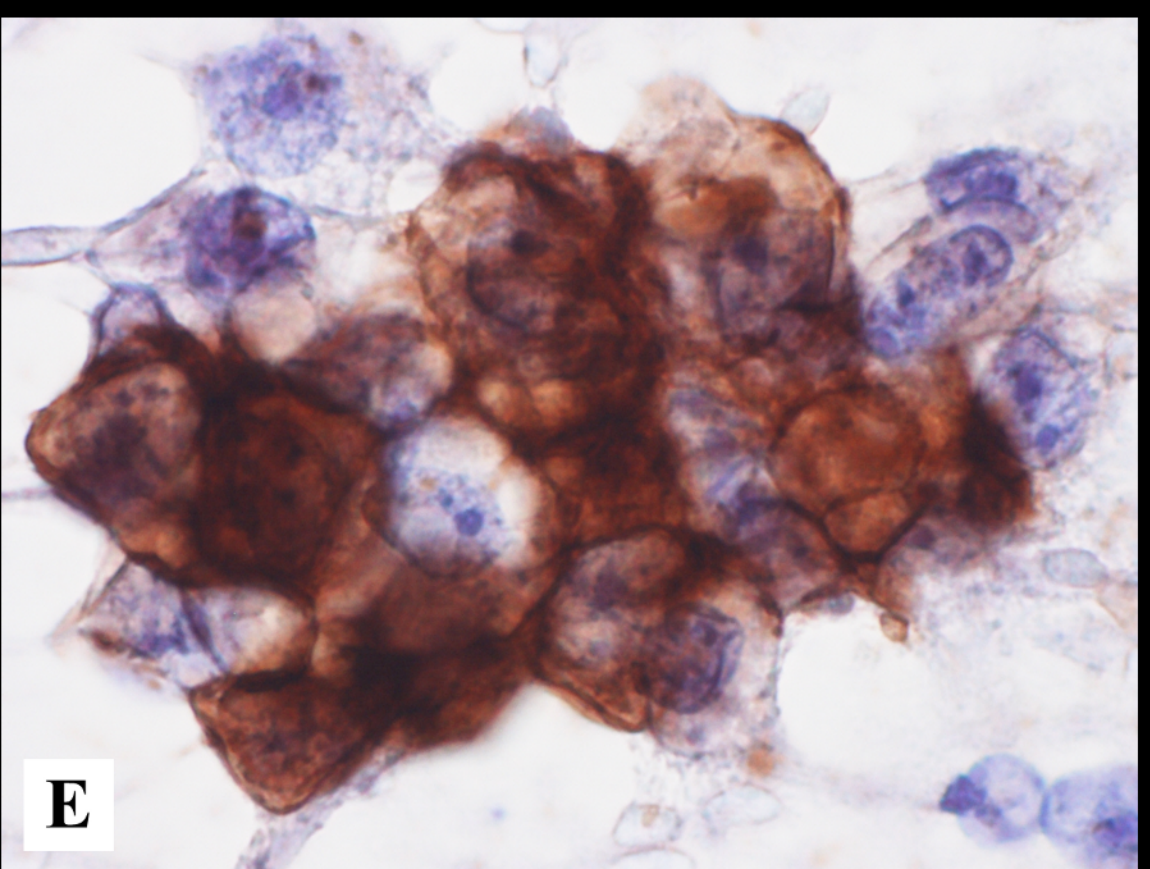
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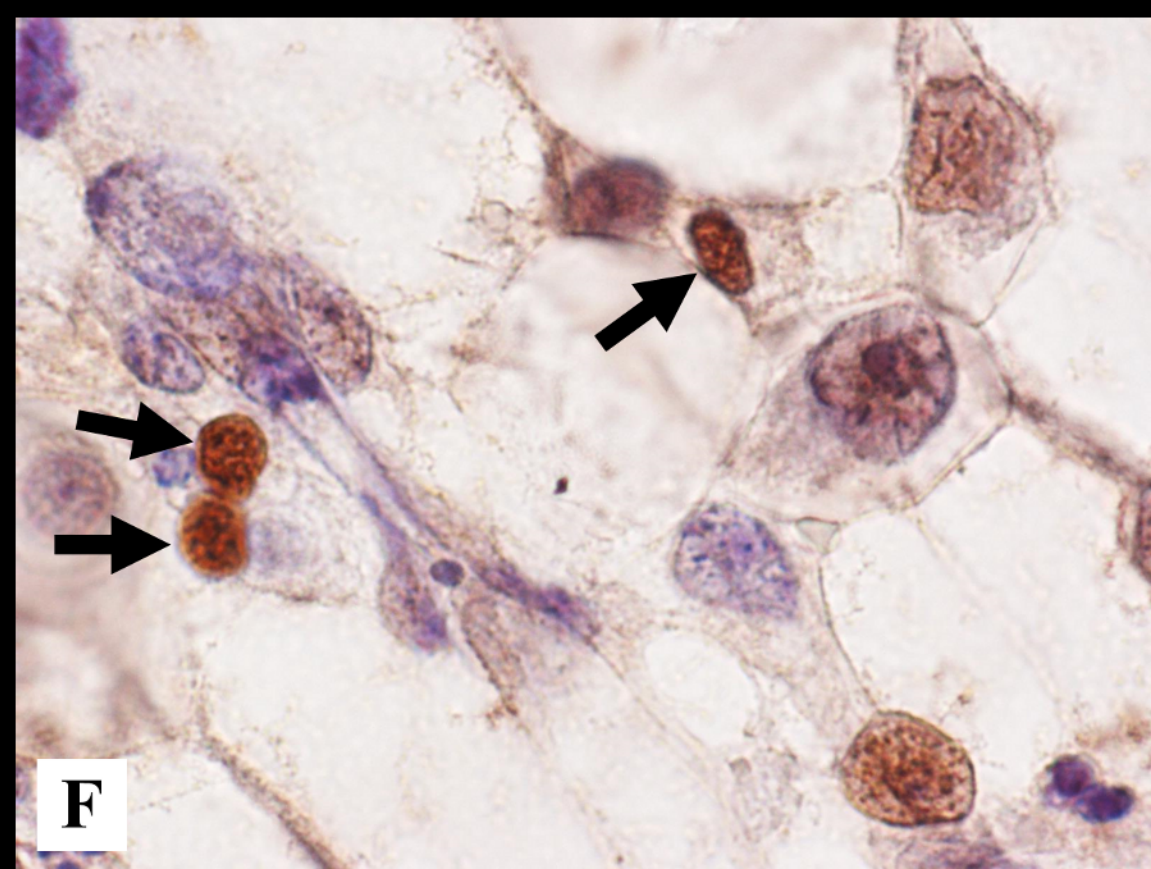
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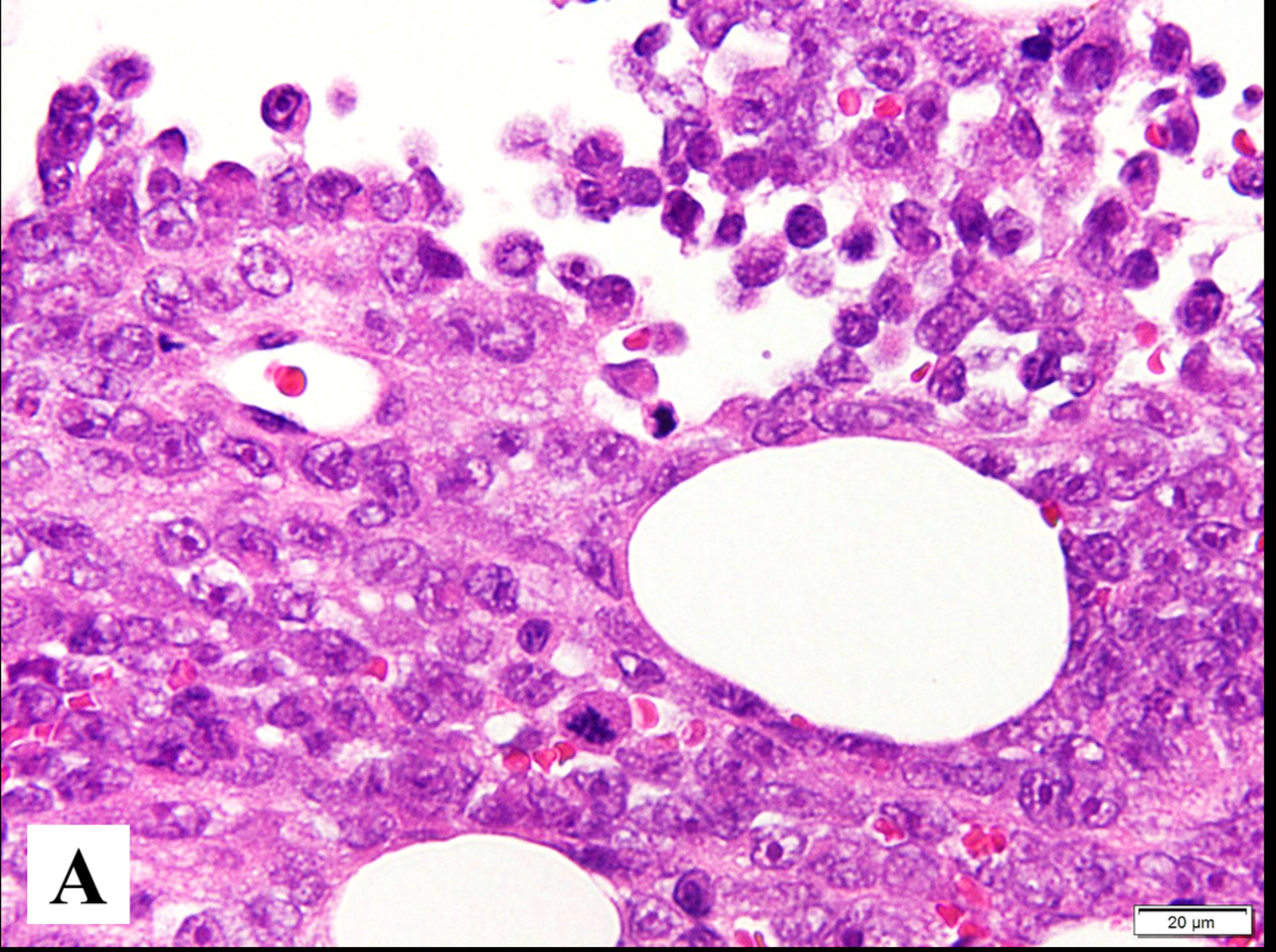
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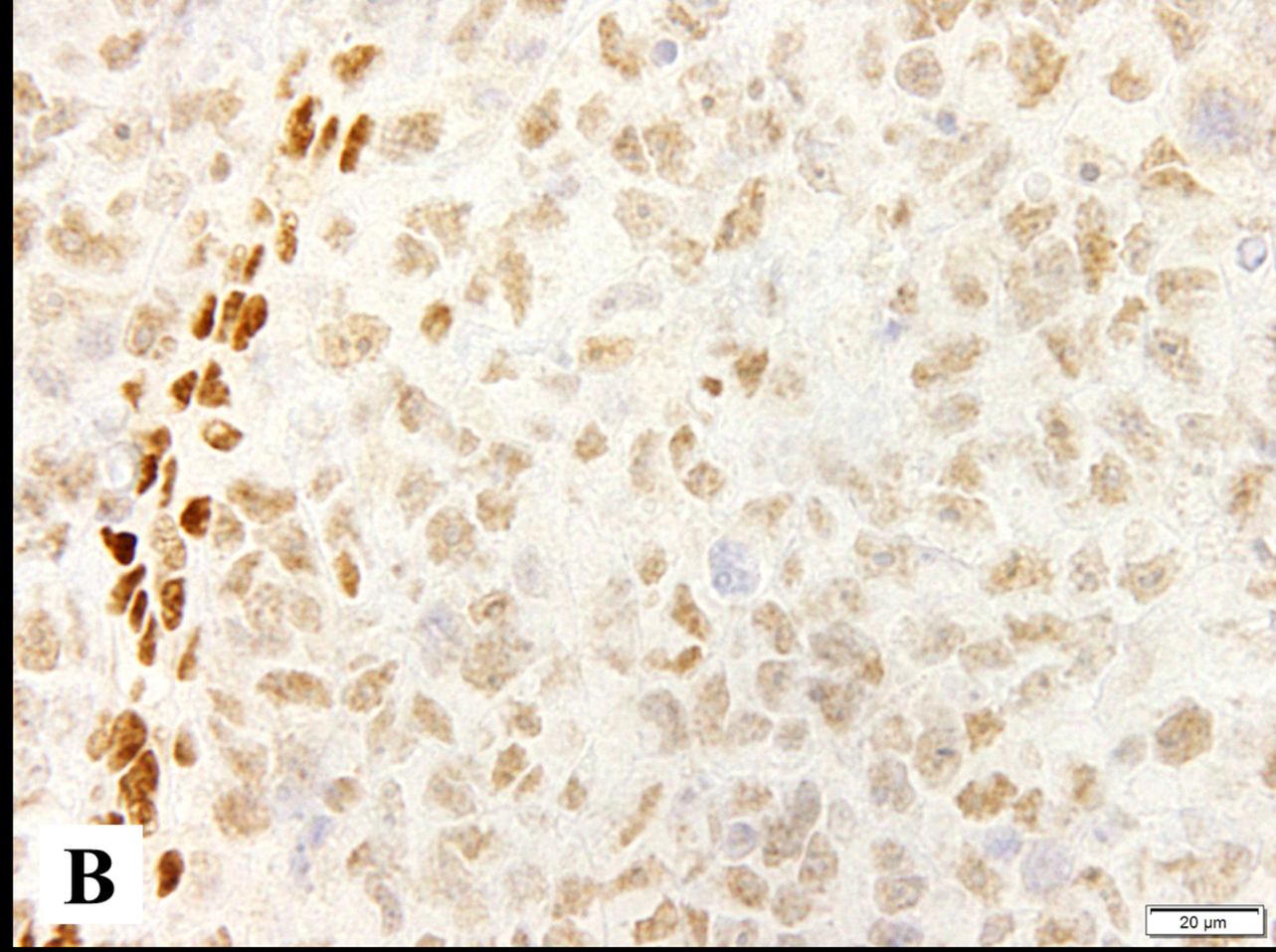
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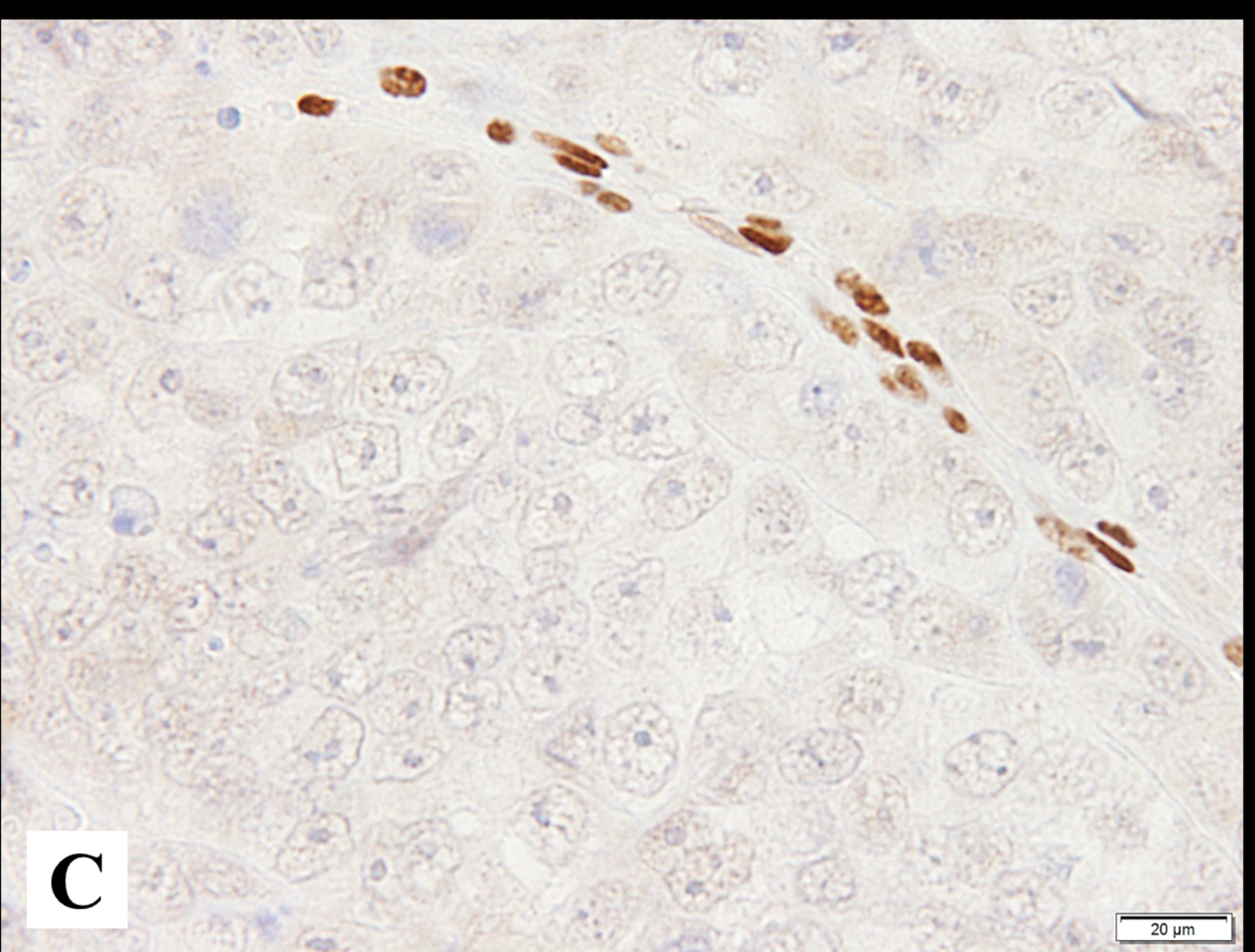
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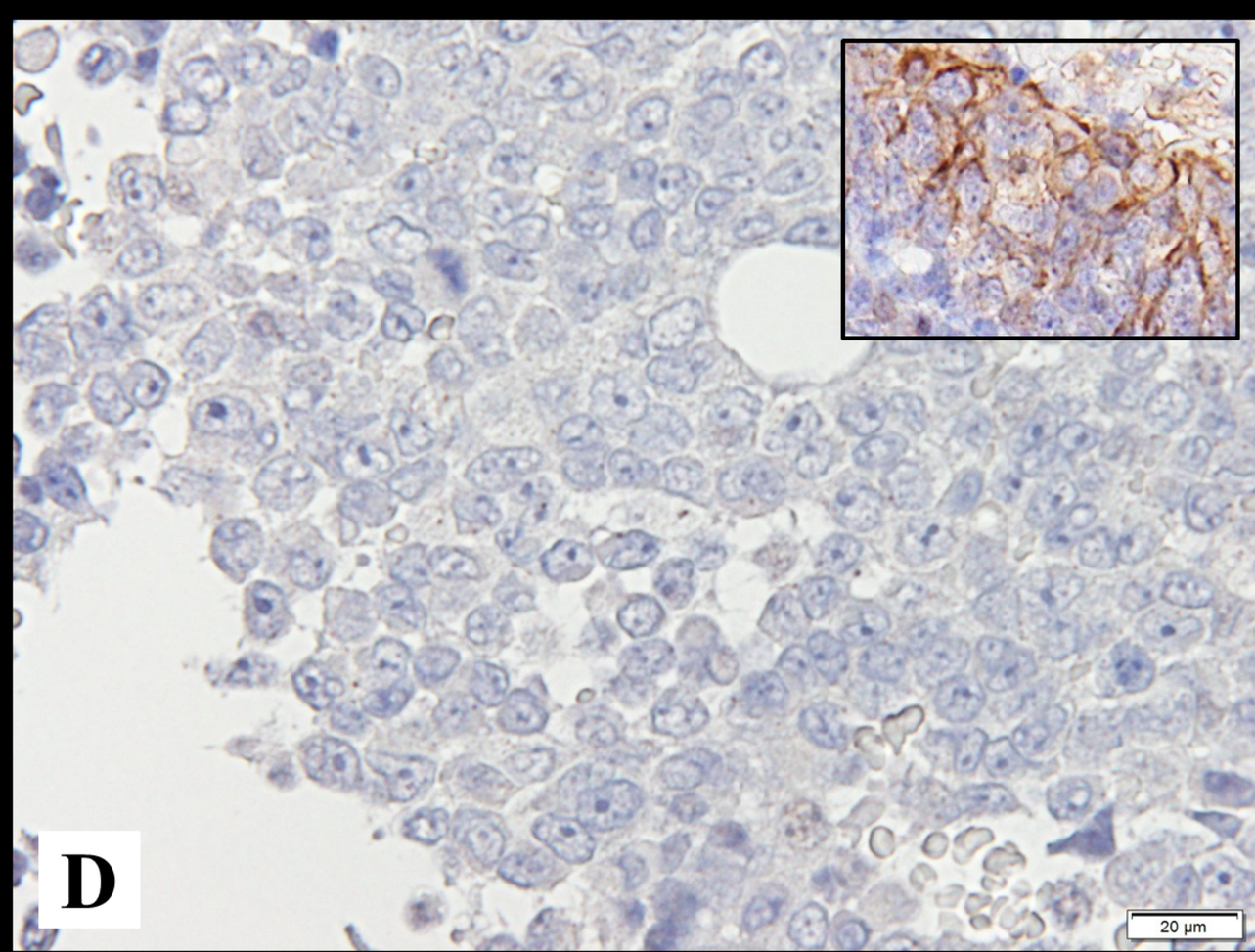
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Table 1 Antibodies and staining conditions for immunocytochemistry

Antibody	Clone	Manufacturer	Dilution	Antigen retrieval
Cytokeratin	CAM5.2	BD Biosciences, San Jose, CA	Prediluted	None
CD34	NU-4A1	Nichirei, Tokyo, Japan	1:100	None
BRG1/SMARCA4	G-7	Santa Cruz Biotechnology, Dallas, TX	1:500	None