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Case Report

The diagnostic significance of reticulin fiber stain in adrenocortical carcinoma biopsies

Research

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Abstract

Aims

To investigate the diagnostic significance of reticulin fiber stain in adrenocortical carcinoma biopsies.

Material and Methods

Adrenal mass biopsy specimens were collected at Sun Yat-sen University Cancer Center from November 2008 to July 2021. The 21 specimens included 19 cases of adrenal cortical carcinoma (ACC) and 2 cases of normal adrenal cortical tissue. Their reticulin fiber stain features were evaluated histologically and immunohistochemically（IHC, and the relevant literature was reviewed.

Results

The nuclei of ACC cancer cells stained intensively dark and varied in size. ACC presented with different degrees of nuclear atypia, as well as mitotic figures, focal necrosis and giant tumor cells. Eosinophilic cytoplasm was frequent, and only seldom transparent. The ACC cells grew in nested, cord-like or diffuse configuration. To the

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contrast, the 2 cases of normal adrenal cortex displayed with partially transparent, partly eosinophilic cytoplasm and small nuclei. Four ACC cases (4/19) were positive for cytokeratin (CK), 6 cases (6/19) positive for Vimentin, 8 cases (8/19) positive for Inhibin-a, and 7 cases were positive (7/19) for Melan-A. Seven cases (7/19) were positive for SF-1, 11 cases (11/19) for Synaptophysin (Syn) and 4 cases (4/19) for CD56. The Ki-67 index ranged from 2% to 90%. The reticulin fiber stain of the 2 cases with normal adrenal cortex revealed that the reticular fibers were regularly arranged. They completely wrapped the adrenal cortex cells. The thickness of the complete reticular fibers was moderate and uniform. The reticular fibers of all 19 ACC cases were incomplete and damaged (19/19), and displayed with discontinuity, breakage, disorder, sparseness or disappearance.

Conclusion

Reticulin fiber stain is considered to be a helpful tool and to improve the diagnostic accuracy of adrenal cortical carcinoma.

Keywords: adrenocortical carcinoma, reticulin fiber stain, transcutaneous biopsy

Introduction

Adrenocortical carcinoma (ACC) is a rare malignant tumor that originates from adrenocortical cells. ACC develops rapidly and possesses a high degree of malignancy and poor prognosis.

Most cases are sporadic, although some cases are associated with genetic syndromes such as the Li-Fraumeni syndrome (TP53 gene mutation), Lynch syndrome (mismatch repair gene mutation) and Beckwith-Wiedeman syndrome.[1] ACCs are usually diagnosed in advanced stages with distant metastases. The 5-year mortality rate is as high as 75%-90%.[2] Despite the application of mitotane-based therapy, the mortality rate remains still high.[3]

Accurate diagnosis of ACC is always the first step to successfully treat patients with malignant tumors. However, the differential diagnosis of ACC remains a challenge for clinicians and pathologists.[4]

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At present, the diagnostic accuracy of transcutaneous adrenal mass biopsies is limited in clinical practice. In addition, a puncture may cause needle-track metastasis.

Therefore, some studies suggest that adrenal transcutaneous biopsies and aspirations posses potential hazards and are not really helpful for the diagnosis of ACC.[5]

Clinicians often perform adrenal mass transcutaneous biopsies only in late or generalized tumor stages, or when surgical treatment is no longer possible because of technical problems.[6]

Tissue based diagnosis, for example biopsy and fine needle aspiration are a useful tool to diagnose solid malignancies such as ACC and might even guide the patient's subsequent treatment.

However, in actual work, we found that the limited tissue size of biopsies and the low frequency of malignancies prevent the application of the Weiss Score which is a useful tool in surgery in general.

Tissue based diagnosis including PCR tools are harmless and permit an accurate and reliable differentiation between benign and malignant solid tumors. Consecutively, pathologists try to work out fast and reliable as well as economically satisfying techniques in the assessment of malignancies such as ACC.

This article aims to explore the diagnostic significance of reticulin fiber stain in transcutaneous biopsy specimens for ACC.

Ethics, Material and Methods

The study has been approved by the Institute Research Medical Ethics Committee of Sun Yat-sen University Cancer Center, Guangzhou, China.

CASE SELECTION

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We thorough re-analyzed fine needle aspirates and transcutaneous biopsies which were sent to the Department of Pathology, Sun Yat-sen University Cancer Center. Guangzhou from November 2008 to July 2021. The clinical history and microscopic morphology were analyzed in 19 ACC cases after confirming that the results of immunohistochemistry are consistent with the adrenal gland tumor origination.

The case collection included 15 males and 4 females, 1 ??? - 72 years old, (mean of 40 years). Two cases (women, 39 years and 53 years old displayed with normal adrenal gland tissue and served for control. The first case, a 39 years old patient suffered from Lung cancer, and her Pet-CT scan suggested a hypermetabolic focus of the adrenal gland. Only normal adrenal tissue was seen in her transcutaneous biopsy. Re-examination of the CT findings resulted in the diagnosis of benign hyperplasia. The patient subsequently underwent radical lung cancer resection at the stage pT2N0M0.

A large retroperitoneal mass was clinically detected in the second patient. A transcutaneous biopsy should clarify the diagnosis and guide the treatment. However, only normal adrenal tissue was seen in the biopsy specimen.

After a short time, the patient underwent surgery and an ACC was stated in the postoperative diagnosis.

A total of 5 out of the 19 patients underwent radical resection of adrenal tumors. Follow-up data of the 19 ACC cases were available for 8 patients. 2 patients died.

IMMUNOHISTOCHEMISTRY AND HISTOCHEMISTRY

The biopsy specimens were fixed with 3.7% formaldehyde, routinely dehydrated, paraffin embedded, and cut in 4 µm serial sections. HE staining and immunohistochemical (IHC) EnVision staining were carried out.

The applied antibodies include CK(clone AE1/3, 1:50 dilution; Dako, Carpinteria, CA) Vimentin (clone V9, diluted 1:250, Dako Cytomation, Glostrup, Denmark)), Inhibin-a (clone R1, diluted 1:75; Diamedix, Miami, FL, USA), Melan-A (clone A103, diluted 1:50; Dako Cytomation, Glostrup, Denmark), SF-1(clone SRC-1, diluted 1:75, Cell Signaling,

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Danvers, MA, USA), Syn (clone SY38, diluted 1:150; Dako Cytomation, Glostrup, Denmark), CD56(clone 123C3, diluted 1:150, Dako Cytomation, Glostrup, Denmark)), Ki-67(clone MIB-1,diluted 1:100 , Dako Cytomation, Glostrup, Denmark)).

A commercially available silver impregnation-based kit (Bio Optica, Milan, Italy) was applied to analyze the status of the reticulin fiber framework.

Results

HISTOLOGICAL MORPHOLOGY

The nuclei of ACC cancer cells stained intensively dark and varied in size. ACC presented with different degrees of nuclear atypia, as well as mitotic figures, focal necrosis and tumor giant cells. Eosinophilic cytoplasm was frequent, and only seldom transparent. The ACC cancer cells grew in nested, cord-like or diffuse formations (Figure 1, 3, 5, 7).

IMMUNOHISTOCHEMISTRY

The obtained results are summarized in table 1. Among the 19 ACC patients, 4 cases (4/19) were positive for CK. 6 cases (6/19) were positive for Vimentin. 8 cases (8/19) were positive for Inhibin-a, and 7 cases (7/19) were Melan-A positive. 7 patients (7/19) were positive for SF-1. 11 patients (11/19) were Syn positive and 4 patients (4/19) were CD56 positive. The Ki-67 index of the patients ranged from 2% to 90%.

Table 1 Immunohistochemical results

Antiboby	Positive	Negative
CK	4/19(21%)	15/19(79%)
Vimentin	6/19 (32%)	13/19 (68%)
Inhibint-a	8/19 (42%)	11/19 (58%)
Melan-A	7/19 (37%)	12/19 (63%)

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SF-1	7/19 (37%)	12/19 (63%)
Syn	11/19 (58%)	8/19 (42%)
CD56	4/19 (21%)	15/19 (79%)
Ki67	2%~90%	

RETICULIN FIBER STAIN

The reticulin fiber stain of the 2 normal adrenal cortex cases showed normal regularly arranged position of the reticulin fibers which completely wrap the adrenal cortex cell nests. The thickness of the fibers was moderate and uniform.

The 19 cases of ACC displayed with incomplete and damaged reticulin fibers (19/19). Discontinuity, breakage, disorder, sparseness, or disappearance are characteristic for this malignancy (Figure 2, 4, 6, 8).

Discussion

Postoperative tumor mass diagnosis of ACC mainly depends on the microscopic morphology, including the Weiss Score and comprehensive IHC analysis.[6] The frequently used morphological diagnostic criteria of the Weiss Score include nine indicators: nuclear atypia, mitotic index (thresholds in high power fields), presence of atypical mitoses, percentage of clear cells / all tumor cells, tumor growth pattern, tumor necrosis, tumor cell invasion in venes, sinus spaces, and capsules.

Although the Weiss Score is a clear-cut and strict morphological diagnosis standard, its application is not ideal after years of practice in surgical pathology.

The reason is, that too many items seem to be involved, and that features such as nuclear atypia, pathological mitosis, diffuse structure, necrosis, and capsular invasion are highly subjective, of poor reproducibility and week consistency in general. The specificity and sensitivity of these features are far from 100%.[7]

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Several authors reported new IHC tools to improve the diagnosis and treatment of adrenocortical neoplasms recently.

For example, Schmitt A et al. proposed that the combination of IGFII and MIB1 IHC results could reliably distinguish between benign and malignant adrenocortical neoplasms.[8] The clinical significance of this proposal still remains to be verified.

In 2009, Volante et al. first described an extension of this technique. The diagnosis of ACC should be consistent with the presence of reticulin fiber damage plus at least one of the following three items: number of mitotic figures (above 5 per 50 nuclei in high-power fields), necrosis of the lesion or vascular invasion of identified tumor cells. They reported that this diagnosis model is of 100% specificity and sensitivity and that reticular fiber destruction possesses 96% specificity and 100% sensitivity in the diagnosis of ACC. This diagnostic model is called "Reticulin Algorithm (RA)". [9]

In 2013, Duregon et al. analyzed the results of the reticulin fiber stain in 245 cases and confirmed that RA was indeed a fast and convenient method to diagnose or confirm ACC.[10]

These studies demonstrate that the destruction of reticulin fibers is a reliable tool for ACC diagnosis. Especially in RA reticulin fiber damage was listed in the first rank of ACC diagnosis.

The reticulin fiber stain has also been proposed as diagnostic tool in other endocrine organs, such as the pituitary gland. Herein, a reticulin fiber stain has been reported an appropriate frozen section technique to differentiate between pituitary adenomas and normal glands.[11, 12]

Kuşku Çabuk et al. described the diagnostic significance of reticulin fibers in parathyroid diseases.[13]

In summary. the diagnostic significance of reticulin fibers in ACC has been widely confirmed; however in gross specimens after surgery only.

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The question remains, how significant is the role of reticulin fiber destruction in transcutaneous adrenal aspirations and biopsies?

As mentioned previously, the Weiss Score standard requires sufficient material and possesses too many items. Its applicability is not satisfying even in surgical specimens. Therefore, transcutaneous biopsies are probably not suitable to apply the Weiss Score in ACC problems.

Our study reports that the reticulin fiber stain displays with incomplete, missing, damaged or destroyed reticulin fibers in 19 transcutaneous biopsy cases of ACC. This result might encourage surgical pathologists to diagnose or at least to suggest ACC, if transcutaneous biopsies display with altered or destroyed reticulin fibers and if the diagnosis ACC is consistent with the IHC findings.

Thee applied staining technique is simple, economical and fast. In our study, the proposed ACC could be confirmed by 5 /19 patients who underwent radical surgery. The 14 patients without surgery received comprehensive treatment. Although all cases in our study showed a severe damage of the investigated reticular fibers, our number of cases remains small. Our results should encourage surgical pathologists to confirm or to refine our data be additional studies.

In summary, reticulin stain of transcutaneous biopsies displayed with severe reticulin fiber destruction. It is an effective, simple and economical tool to obtain a fast and reliable diagnosis of ACC. It can be performed, standardized and interpreted in nearly all pathology departments. It possesses a good clinical applicability and is worthy of promotion.

Author's contributions

Suhua Wu & Wanlin Tan: data collection, picture collection, drafted the manuscript; Ping Yang: guiding experiments; Yijun Zhang: guiding experiments; Mayan Huang: experimental operation; Yun Cao: design this study, revising the manuscript

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Conflict of interest

All authors declare that there is no conflict of interest

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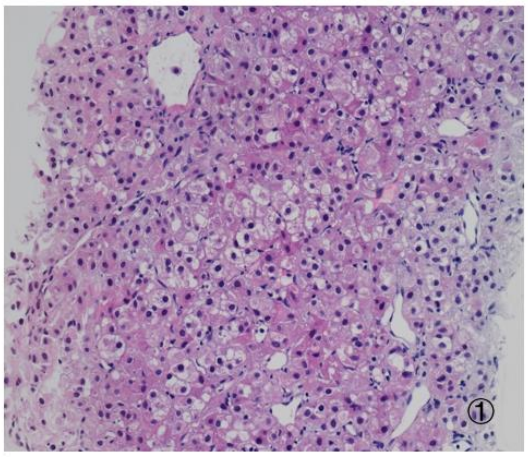

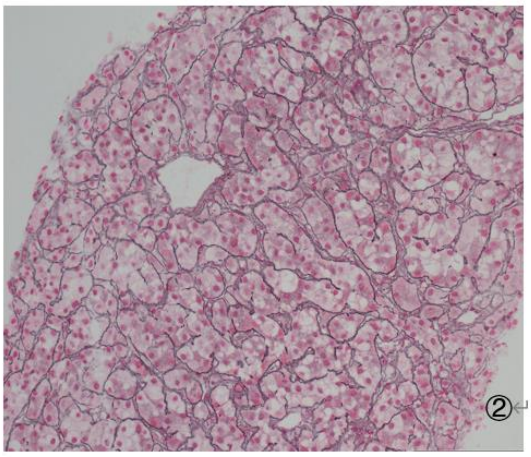

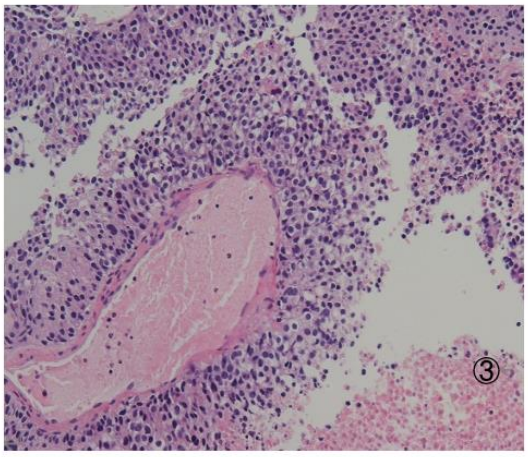

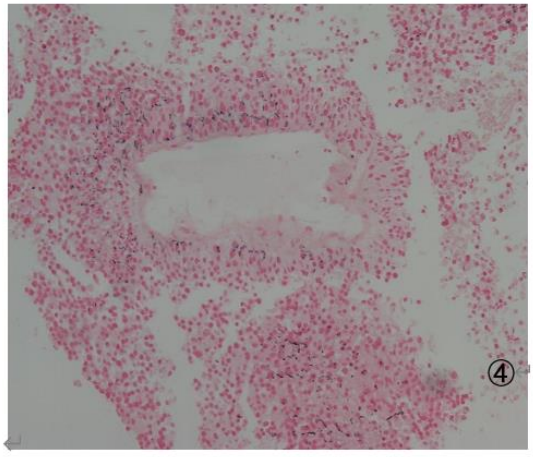

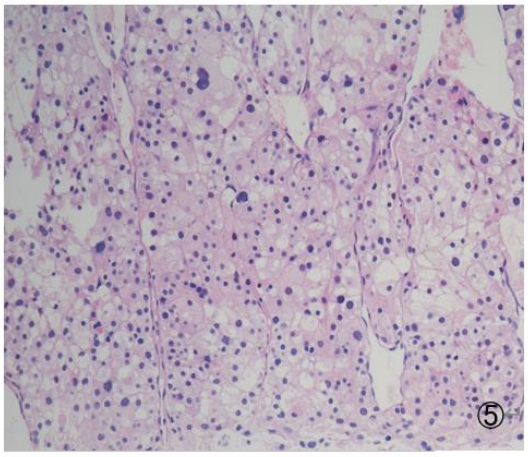

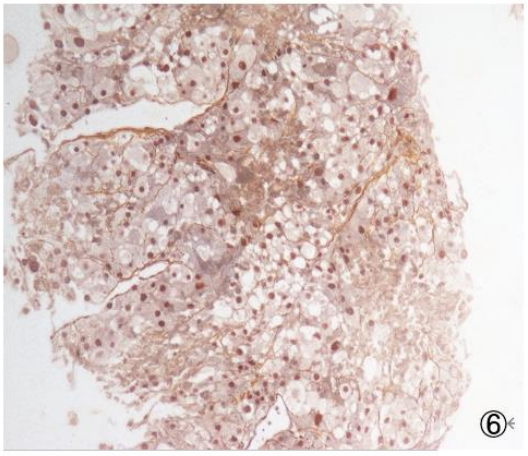

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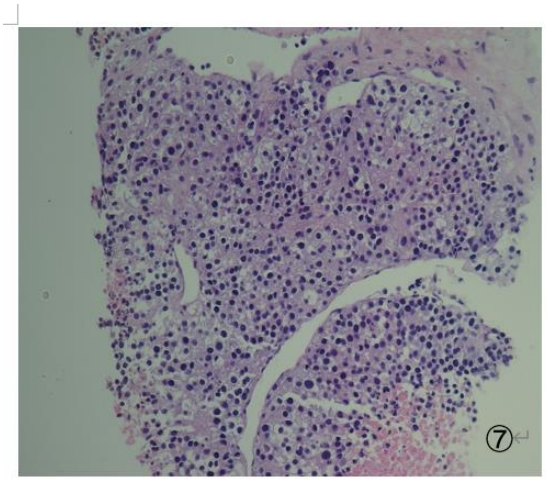

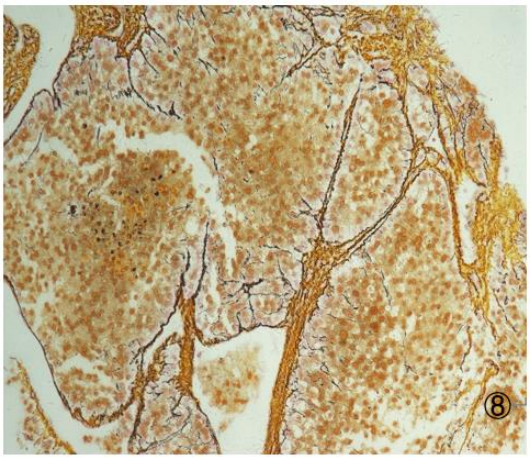

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RETICULIN FIBER STAIN

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The 19 cases of ACC displayed with incomplete and damaged reticulin fibers (19/19). Discontinuity, breakage, disorder, sparseness, or disappearance are characteristic for this malignancy (Figure 2, 4, 6, 8).

Discussion

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For example, Schmitt A et al. proposed that the combination of IGFII and MIB1 IHC results could reliably distinguish between benign and malignant adrenocortical neoplasms.[8] The clinical significance of this proposal still remains to be verified.

In 2009, Volante et al. first described an extension of this technique. The diagnosis of ACC should be consistent with the presence of reticulin fiber damage plus at least one

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of the following three items: number of mitotic figures (above 5 per 50 nuclei in high-power fields), necrosis of the lesion or vascular invasion of identified tumor cells. They reported that this diagnosis model is of 100% specificity and sensitivity and that reticular fiber destruction possesses 96% specificity and 100% sensitivity in the diagnosis of ACC. This diagnostic model is called "Reticulin Algorithm (RA)". [9]

In 2013, Duregon et al. analyzed the results of the reticulin fiber stain in 245 cases and confirmed that RA was indeed a fast and convenient method to diagnose or confirm ACC.[10]

These studies demonstrate that the destruction of reticulin fibers is a reliable tool for ACC diagnosis. Especially in RA reticulin fiber damage was listed in the first rank of ACC diagnosis.

The reticulin fiber stain has also been proposed as diagnostic tool in other endocrine organs, such as the pituitary gland. Herein, a reticulin fiber stain has been reported an appropriate frozen section technique to differentiate between pituitary adenomas and normal glands.[11, 12]

Kuşku Çabuk et al. described the diagnostic significance of reticulin fibers in parathyroid diseases.[13]

In summary. the diagnostic significance of reticulin fibers in ACC has been widely confirmed; however in gross specimens after surgery only.

The question remains, how significant is the role of reticulin fiber destruction in transcutaneous adrenal aspirations and biopsies?

As mentioned previously, the Weiss Score standard requires sufficient material and possesses too many items. Its applicability is not satisfying even in surgical specimens. Therefore, transcutaneous biopsies are probably not suitable to apply the Weiss Score in ACC problems.

Our study reports that the reticulin fiber stain displays with incomplete, missing, damaged or destroyed reticulin fibers in 19 transcutaneous biopsy cases of ACC. This

result might encourage surgical pathologists to diagnose or at least to suggest ACC, if transcutaneous biopsies display with altered or destroyed reticulin fibers and if the diagnosis ACC is consistent with the IHC findings.

Thee applied staining technique is simple, economical and fast. In our study, the proposed ACC could be confirmed by 5 /19 patients who underwent radical surgery. The 14 patients without surgery received comprehensive treatment. Although all cases in our study showed a severe damage of the investigated reticular fibers, our number of cases remains small. Our results should encourage surgical pathologists to confirm or to refine our data be additional studies.

In summary, reticulin stain of transcutaneous biopsies displayed with severe reticulin fiber destruction. It is an effective, simple and economical tool to obtain a fast and reliable diagnosis of ACC. It can be performed, standardized and interpreted in nearly all pathology departments. It possesses a good clinical applicability and is worthy of promotion.

Author's contributions

Suhua Wu & Wanlin Tan: data collection, picture collection, drafted the manuscript; Ping Yang: guiding experiments; Yijun Zhang: guiding experiments; Mayan Huang: experimental operation; Yun Cao: design this study, revising the manuscript

Conflict of interest

All authors declare that there is no conflict of interest

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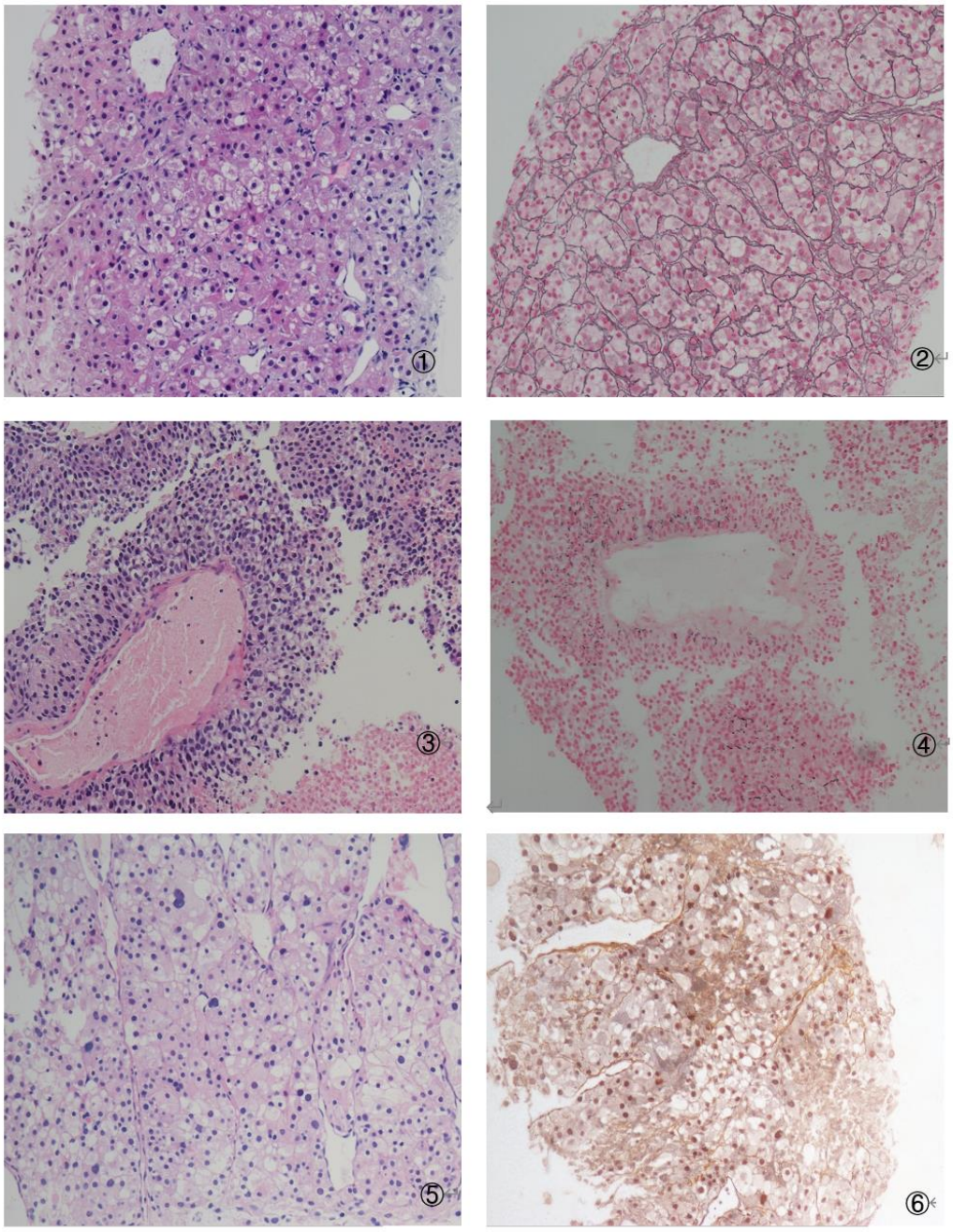
Figures and Legends



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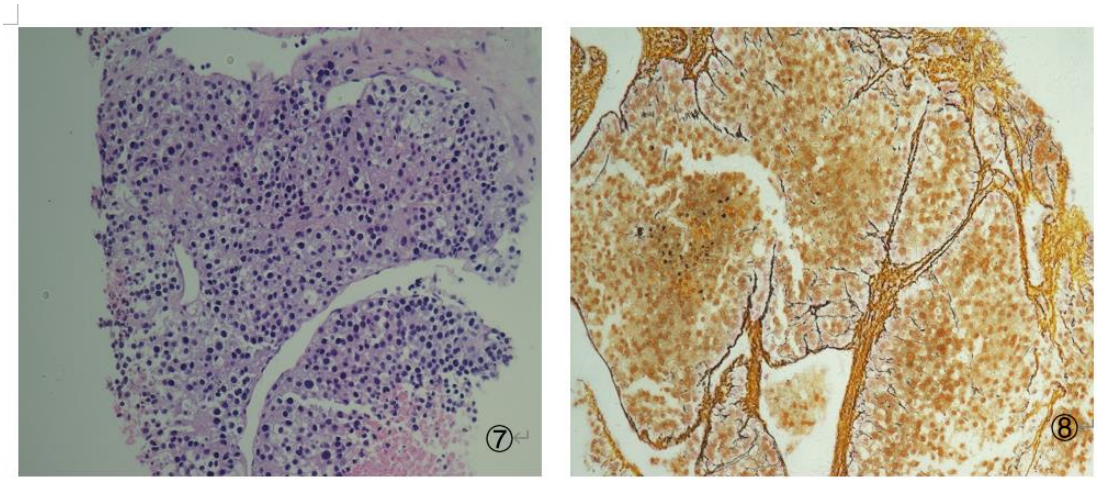

Figure 1: The normal adrenal cortex tissue displays with partially transparent, partly eosinophilic cytoplasm and small nuclei in homogeneous size and pattern.

Figure 2 The reticulin stain of normal adrenal cortex reveals regularly arranged and completely wrapped reticular fibers.

Figure 3: ACC cancer cells possess intensively stained nuclei of great variation in size. Various degrees of nuclear atypia, mitotic figures, focal necrosis and tumor giant cells can be noted.

Figure 4: Large areas of infarct – like necrosis are noted in this case of ACC.

Figure 5: ACC cancer cells represent eosinophilic cytoplasm, multiple and frequently enlarged nuclei.

Figure 6: ACC cancer cells break the original solid reticulin fibers into fragments and consecutively create disorder and total destruction.

Figure 7: The ACC cancer cells grow in solid structures, diffuse pattern and invade vascular walls.

Figure 8: The original reticulin fiber network was not apparent any more in this and all analyzed ACC biopsies (19/19).