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Case Report

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Rapid Intraoperative Immunohistochemistry (RD-IHC) is a Useful Tool in Intraoperative Pathologic Diagnosis

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Abstract

Intraoperative pathologic diagnosis for lesions plays an important role in determining the surgery procedure. Accurate pathological diagnosis could lead to earlier, more effective treatments. But the accuracy of frozen sections pathology diagnosis in controversial cases maybe discount. Most of the time, the surgeon had to wait for the post-operative immunohistochemistry results to decide whether to have a second operation. In our study, rapid intraoperative immunohistochemistry was used for the diagnosis of controversial cases, which greatly improved the diagnostic accuracy and reduced rates of deferred diagnoses. Also, the total procedures of rapid intraoperative immunohistochemistry can be finished within 10 min that allows its general application for intraoperative frozen section diagnosis. In conclusion, rapid intraoperative immunohistochemistry could be a useful tool in intraoperative pathologic diagnosis.

Keywords: RD-IHC; FS; Lung mucinous adenocarcinoma; Lung sclerosing pneumocytoma; Breast sclerosing adenosis; Brain high-grade glioma; Lymphoma

Abbreviations: RD-IHC: Rapid Intraoperative Immunohistochemistry; CT: Computed Tomography; SP: Sclerosing Pneumocytom; FS: Frozen Sections

Background

Frozen Sections (FS) of tumor tissues represent the basis of pathological intraoperative diagnosis and have a significant role in the microscopic analysis of surgery specimens [1]. An uncertain or incorrect pathological diagnosis will have a malicious impact for the patient, which could lead to a second operation or over surgery. Therefore, an accurate intraoperative diagnosis is very important. So far, immunohistochemical staining on FS has been confirmed for several antibodies using manual methods via an improved Envision two-step method. However, this test usually takes about 30 minutes. At present, several methods of Rapid Intraoperative

Immunohistochemistry (RD-IHC), such as EnVision two-step method, fast fluorescence IHC and IHC under alternating current electric field, have been selected for detection in some studies to improve the accuracy of intraoperative pathologic diagnosis [2-4]. In our study, RD-IHC using one-step deactivation procedure, which only took 10 minutes, was used in this study to solve the problem of intraoperative diagnosis of controversial lesions [5]. In the current study, antibodies such as CK(AE1/AE3), CK5/6, P63, P40, Vimentin, GFAP, LCA, syn, Vimentin and CK7 were used to distinguish the controversial diseases, such as lung mucinous adenocarcinoma and bronchial adenoma, lung sclerosing pneumocytoma and invasive adenocarcinoma, breast sclerosing adenosis and invasive cancer, brain high-grade glioma and lymphoma, etc.

Material and Methods

Sample preparation

The frozen tissues were obtained from Department of Pathology, Shandong Provincial Hospital Affiliated to Shandong First Medical University (Jinan, Shandong, China). Ethical approval was obtained from the Shandong Provincial Hospital Affiliated to Shandong First Medical University. The samples included breast, lung or brain carcinoma, sentinel lymph node and resection margin, etc.

Rapid intraoperative immunohistochemistry

After the pathologist checked and examined the tumor tissues or lymph nodes, the frozen lesion tissues were performed at -20℃ using a Leica CM1950 frozen slicer. FS were sliced from each lesion area and routinely fixed for 30s. Then two FS were performed for HE stains, other FS were proceeded for RD-IHC. RD-IHC used direct immunohistochemistry kit and antibody form Mxinda medical technology Co., LTD (Guizhou, China). The antibodies used in this study are CK(AE1/AE3), CK5/6, P63, P40, GFAP, LCA, syn, Vimentin and CK7. The FS were washed via PBS for three times immediately after fixed. Then, they were blocked with blocking buffers for one minute. Next, add RD-IHC antibodies and incubate for three minutes. The DAB solution was then added into object slides for three minutes. Moreover, rinsing with water and adding hematoxylin, and finally dehydrating with 100% ethanol. The whole RD-IHC process lasts about 10 minutes.

Case Presentation

Lung mucinous adenocarcinoma or bronchial adenoma?

In this study, we collected three cases of lung mucinous adenocarcinoma and bronchiolar adenoma that were not easily distinguished by FS. The clinical information of the three cases is shown in **Table 1.** Under the microscope, the main body of the tumor is located in the lung parenchyma outside the bronchioles. The tumor cells grow along the alveolar wall, and there seems to be a layer of basal cells at the base of the mucous cells. Ciliated cells are not readily visible in FS. Such tissue morphology cannot be confirmed in rapid intraoperative diagnosis, and it is necessary to wait for postoperative HE and immunohistochemistry sections to distinguish whether the morphology conformed to mucinous adenocarcinoma or bronchiolar adenoma. RD-IHC staining was performed on the FS using P63 and P40 antibody, and it was found that no positive basal cells were detected under mucous cells in these three cases **(Figure 1).** So, these three lung tumors were all diagnosed lung mucinous adenocarcinoma. Postoperative HE sections and immunohistochemistry results also confirmed this diagnosis **(Figure 2).** In conclusion, the intraoperative FS and RD-IHC staining results can provide reliable evidence for the diagnosis of lung mucinous adenocarcinoma.

Table 1. The clinical information of the three lung tumor cases.

Pathological number	Gender	Age(year)	CT	Size(cm)
S2205303	male	58	solid nodules in the upper lobe of the right lung	0.7x0.6
S2123230	female	46	ground glass nodule in the inferior lobe of the right lung	2.7x1.8
S2123630	male	62	mixed ground glass nodule in the inferior lobe of the left lung	0.5x0.3

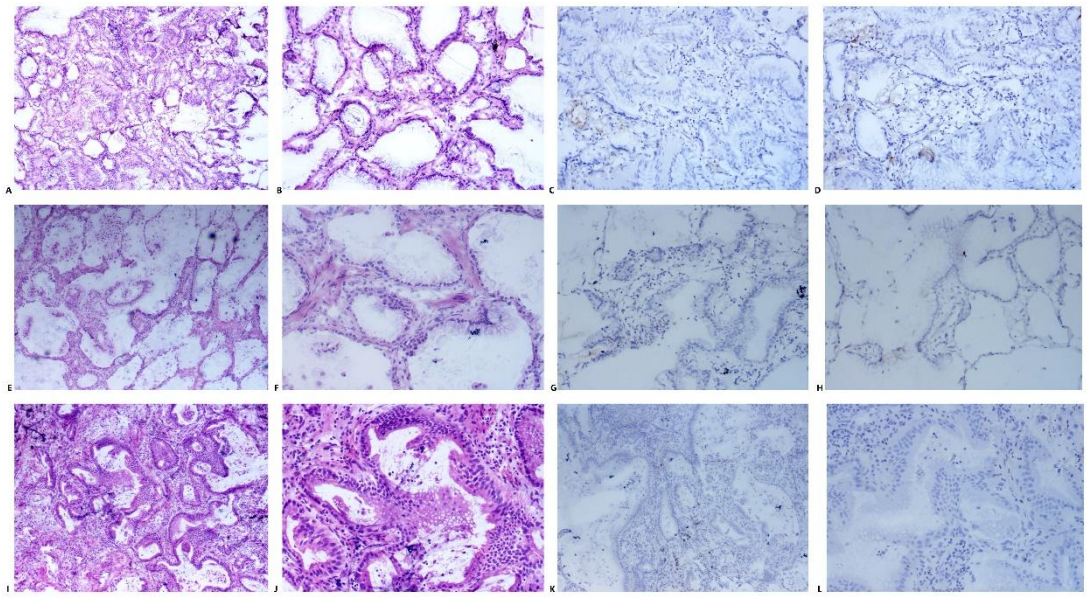

Figure 1: Application of RD-IHC in the diagnosis of lung mucinous tumors.

(A) Intraoperative HE sections of S2205303 (x100); (B) Intraoperative HE sections of S2205303 (x200); (C) Negative immunostaining of P40 (x100); (D) Negative immunostaining of P63 (x100); (E) Intraoperative HE sections of S2123230 (x100); (F) Intraoperative HE sections of S2123230 (x200); (G) Negative intraoperative immunostaining of P40 (x200); (H) Negative intraoperative immunostaining of P63 (x200); (I) Intraoperative HE sections of S2123630 (x100); (J) Intraoperative HE sections of S2123630 (x200); (K) Negative intraoperative immunostaining of P40 (x200); (L) Negative intraoperative immunostaining of P63 (x200).

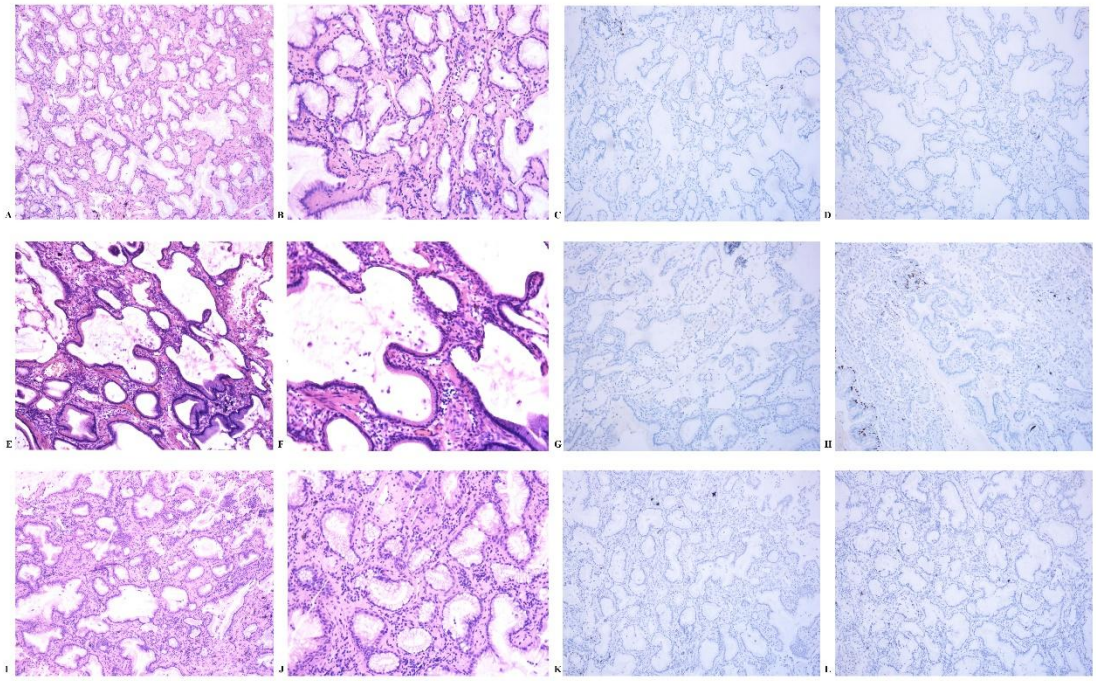

Figure 2: Postoperative HE sections and immunohistochemistry results of three lung mucinous tumors. (A) Postoperative HE sections of S2205303 (x100); (B) Postoperative HE sections of S2205303 (x200); (C) Negative postoperative immunostaining of P40 (x100); (D) Negative postoperative immunostaining of P63 (x100); (E) Postoperative HE sections of S2123230 (x100); (F) Postoperative HE sections of S2123230 (x200); (G) Negative postoperative immunostaining of P40 (x100); (H) Negative postoperative immunostaining of P63 (x100); (I) Postoperative HE sections of S2123630 (x100); (J) Postoperative HE sections of S2123630 (x200); (K) Negative postoperative immunostaining of P40 (x100); (L) Negative postoperative immunostaining of P63 (x100).

Lung sclerosing pneumocytoma or invasive adenocarcinoma?

The pulmonary nodular patient included in this study is a 52-year-old female. A chest Computed Tomography (CT) scan showed an abnormal density and solid mass. Gross examination showed a grayish-white and grayish-red solid cut with a well-defined boundary and a size of 2.8x2.0 cm. Microscopically, alveoli epithelial cells showed atypical hyperplasia papillary pattern, partly grown along the alveolar wall with sclerotic stroma, which were indistinguishable from adenocarcinoma. RD-IHC staining was performed on the FS using Vimentin and CK7 antibody, and it was found that the RD-IHC staining of CK7 was positive in surface cells and Vimentin was positive in round cell components **(Figure 3A-D).** Based on the expression pattern of Vimentin and CK7, pathologist could diagnose lung Sclerosing Pneumocytom (SP) more accurately. Postoperative HE sections and immunohistochemistry results were similar with the RD-IHC results **(Figure 3E-H).**

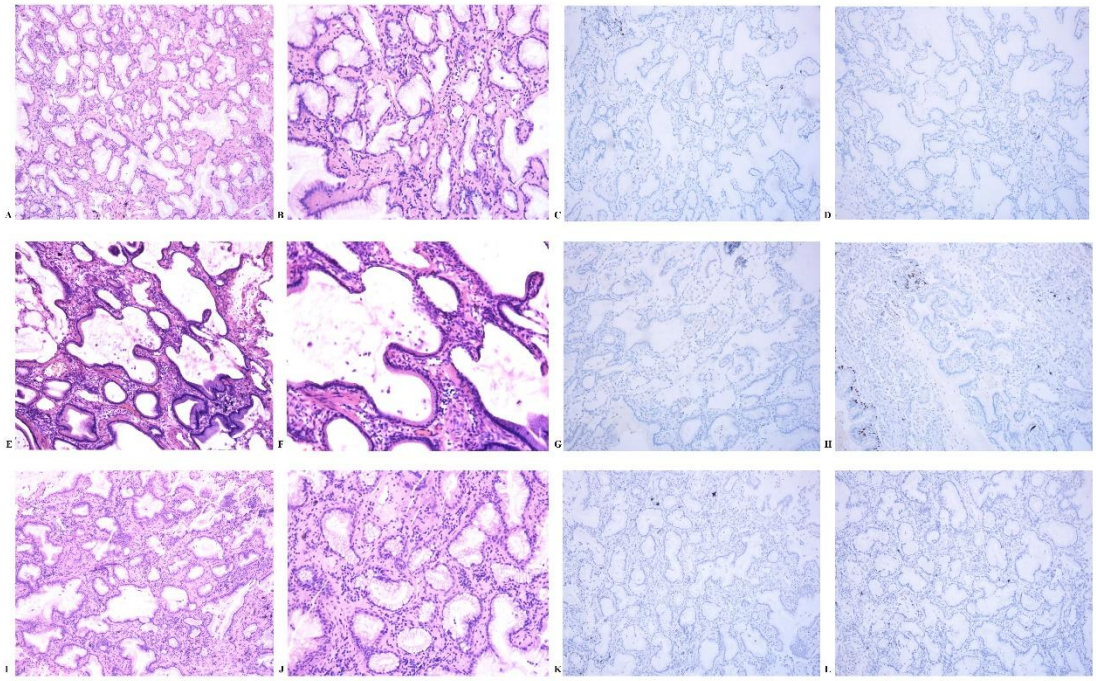

Figure 3: Application of RD-IHC in the diagnosis of lung sclerosing pneumocytoma.

(A) Intraoperative HE sections (x100); (B) Intraoperative HE sections (x200); (C) Positive intraoperative immunostaining of Vimentin in round cell components (x100); (D) Positive intraoperative immunostaining of CK7 in surface cells (x100). (E) Postoperative HE sections (x100); (F) Postoperative HE sections (x200); (G) Positive postoperative immunostaining of Vimentin in round cell components (x100); (H) Positive postoperative immunostaining of CK7 in surface cells (x100).

Brest sclerosing adenosis or invasive cancer?

One 46-year-old female of breast neoplasms was included in this study. Ultrasonography examination showed a 4A nodule. The results obtained from microscopy observation showed that ductal epithelial hyperplasia, sclerotic stroma and fasciculus tumor cells could be screen in this case. Also, scattered tumor cells could be screened near the ducts. Myoepithelial cells cannot be distinguished in these areas and invasive carcinoma cannot be ruled out directly. RD-IHC staining using P63 and CK5/6 antibodies were performed on the FS, and it was found that myoepithelial cells could be found in these areas **(Figure 4A-D).** Postoperative immunohistochemistry results also supported this diagnosis **(Figure 4E-H). In** conclusion, the intraoperative FS and RD-IHC staining results can provide reliable evidence for the diagnosis of breast sclerosing adenosis, effectively avoiding excessive surgery.

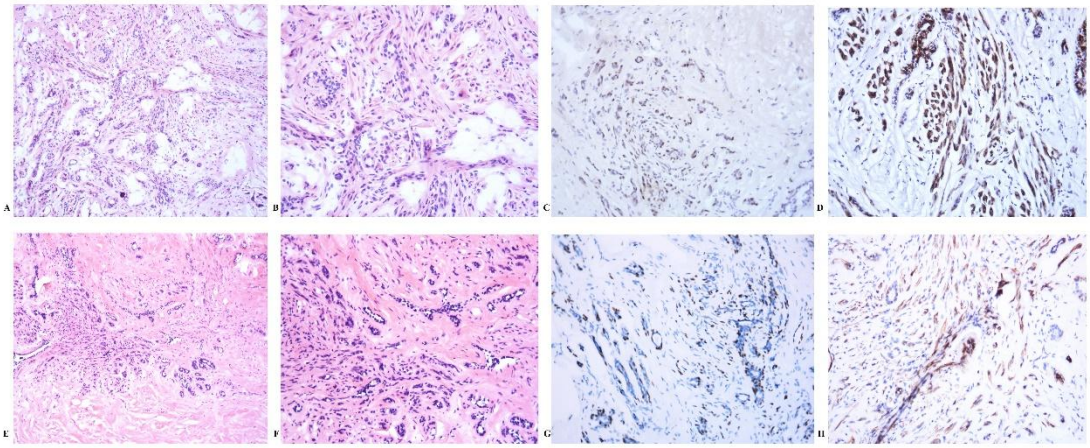

Figure 4: Application of RD-IHC in the diagnosis of breast sclerosing adenosis.

(A) Intraoperative HE sections (x100); (B) Intraoperative HE sections (x200); (C) Positive intraoperative immunostaining of P63 in lesion (x200); (D) Positive intraoperative immunostaining of CK5/6 in lesion (x200). (D) Postoperative HE sections (x100); (E) Postoperative HE sections (x200); (F) Positive postoperative immunostaining of P63 in lesion (x200); (G) Positive postoperative immunostaining of CK5/6 in lesion (x200).

Brain high-grade glioma or lymphoma?

A 56-year-old female (S2203315) and a 54-year-old male (S2124149) were included in our study, which were diagnosed frontal lobe mass via brain MR examination. Microscopically, the hyperplastic tumor cells infiltrated the brain tissue, with hyperchromatic nuclei and frequent mitoses. It is difficult for pathologists to distinguish high-grade glioma or lymphoma. RD-IHC staining using GFAP and LCA antibody were performed on the FS. The results shown that the GFAP was strong positively expressed in cytoplasm in glial cells in S2124149, and negatively expressed in S2203315, while LCA had the opposite expression pattern to GFAP in both two cases **(Figure 5).** So, S2203315 was diagnosed lymphoma and S2124149 was diagnosed high-grade glioma. Postoperative immunohistochemistry sections get the same results with RD-IHC **(Figure 6).**

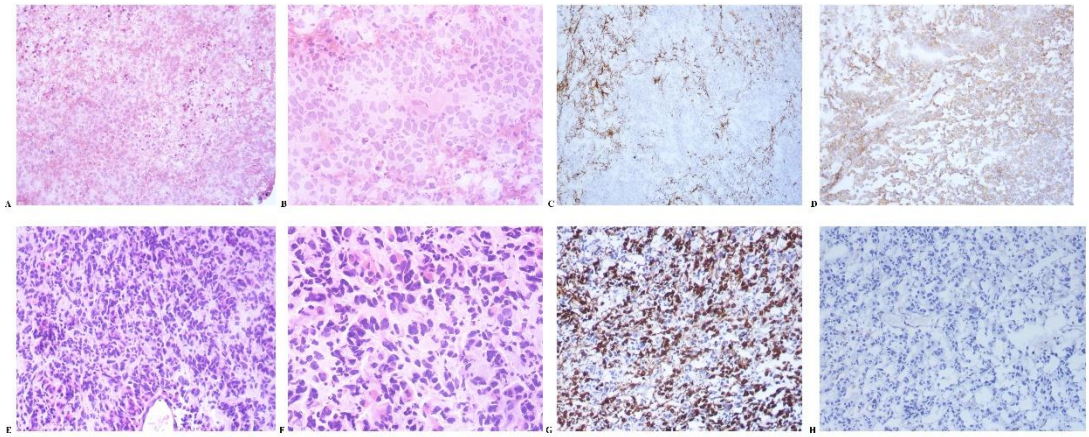

Figure 5: Application of RD-IHC in the diagnosis of brain high-grade glioma or lymphoma.

(A) Intraoperative HE sections of S2203315 (x200); (B) Intraoperative HE sections of S2203315 (x400); (C) Negative immunostaining of GFAP in tumor cells (x200); (D) Positive immunostaining of LCA in tumor cells (x200); (E) HE sections of S2124149 (x200); (F) Intraoperative HE sections of S2124149 (x400); (G) Positive immunostaining of GFAP in tumor cells (x200); (H) Negative immunostaining of LCA in tumor cells (x200).

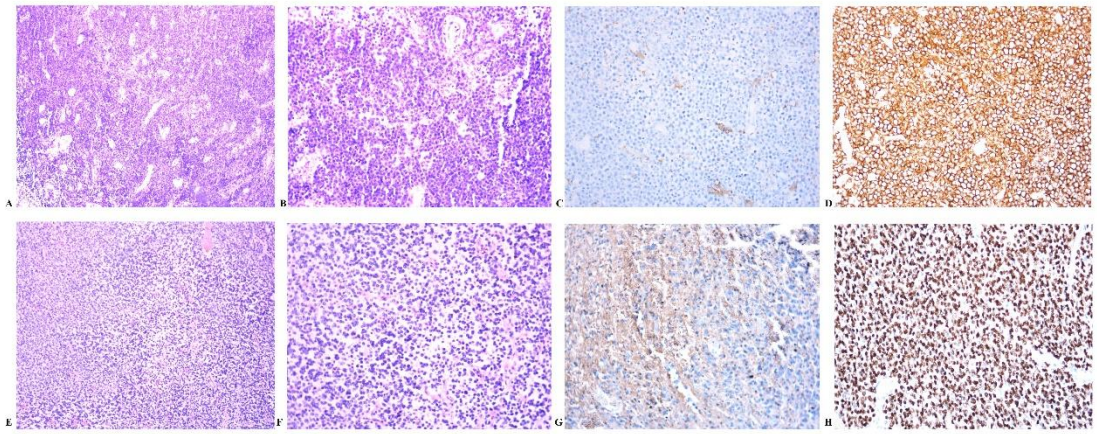

Figure 6: Postoperative HE sections and immunohistochemistry results of brain high-grade glioma or lymphoma.

(A) Postoperative HE sections of S2203315 (x100); (B) Postoperative HE sections of S2203315 (x200); (C) Negative postoperative immunostaining of GFAP in tumor cells (x200); (D) Positive postoperative immunostaining of CD20 in tumor cells (x200); (E) Postoperative HE sections of S2124149 (x200); (F) Postoperative HE sections of S2124149 (x400); (G) Positive postoperative immunostaining of GFAP in tumor cells (x200); (H) Positive postoperative immunostaining of Oligo-2 in tumor cells (x200).

Is cancer seen inside resection margin or lymph node?

A 55-year-old male patient with lung tumor in left lower lobar bronchus was diagnosed lung small cell carcinoma, and the surgeons sent bronchial resection margin for intraoperative pathologic diagnosis. Microscopically, diffuse cells with hyperchromatic nuclei could be seen around the bronchi. It is uncertain to diagnosis to cancer cells or lymphocytes. So syn and LCA antibody were applied for RD-IHC staining in the FS.

As results, LCA was strong positively expressed, while syn was negatively expressed in these diffuse cells **(Figure 7).** Finally, bronchial resection margin was diagnosed “a negative resection margin”.

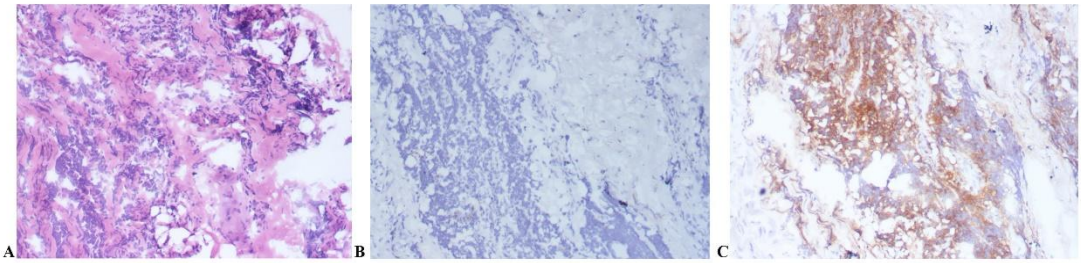

Figure 7: Application of RD-IHC in the diagnosis of cancer inside resection margin or lymph node. (A) Intraoperative HE sections (x200); (B) Negative intraoperative immunostaining of syn in suspicious cells (x200); (C) Positive intraoperative immunostaining of LCA in suspicious cells (x200).

The CK(AE1/AE3) antibody could also be used in pathological diagnosis of lymph node metastasis. When lymph nodes have small metastases, RD-IHC staining using CK(AE1/AE3) antibody could accurately identify cancer cells **(Figure 8).**

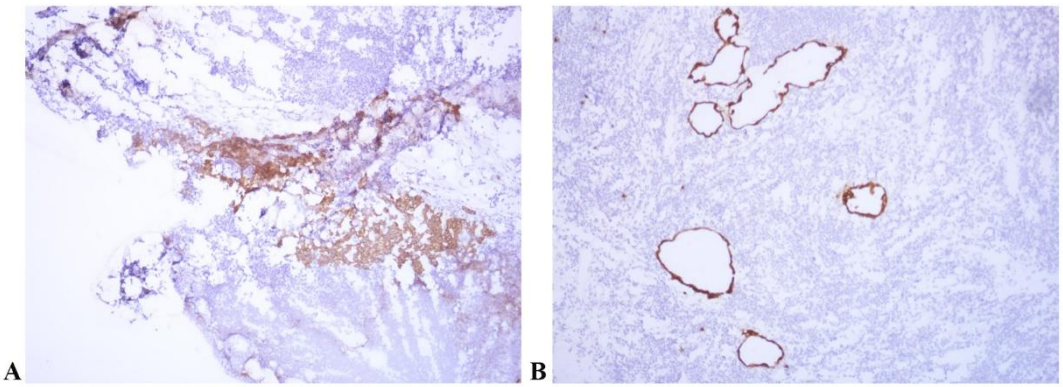

Figure 8: Application of RD-IHC in the diagnosis of small metastases in lymph nodes. (A) Positive intraoperative immunostaining of CK(AE1/AE3) in sentinel lymph nodes of the breast (x100); (B) Positive intraoperative immunostaining of CK(AE1/AE3) in central lymph nodes of thyroid (x100).

Discussion and Conclusions

Bronchiolar adenoma is most easily confused with invasive mucinous adenocarcinoma, especially those with non-papillary proximal bronchiolar adenoma with prominent mucinous features **[6,7].** Because they both have the growth pattern along the alveolar wall, a large number of extracellular mucus and mucus cells can appear discontinuous jump growth mode, and the cells of both could be mild-shaped, which makes the two have amazing similarity in organizational morphology. Due to the obvious differences in the treatment principles and prognosis of the two diseases, it is significant to distinguish them accurately. The key differentiator is the presence of continuous basal cell layers and ciliated cells. In FS, it is easily to identify multilayer basal cells, while it is difficult to identify single layer basal cells. Using P63 and P40 antibody for RD-IHC staining can

clearly recognize basal cells and avoid misdiagnosis and missed diagnosis. The lung SP often presented mass or nodules in CT examination **[8,9].** However, the morphology of is diverse in histopathology. At present, the imaging examinations and clinical features lack specificity partly because the lower rates of SP. Also, it is difficult to puncture typical lesions via biopsy. Therefore, intraoperative pathologic diagnosis is often needed to determine the surgical method and resection scope. But the accuracy of intraoperative pathologic diagnosis of SP is low, which was easily misdiagnosed as malignant lung tumor, leading to delay treatment. Microscopically, SP showed two kinds of cells (surface and round cell components) and four kinds of structures (papillary, bleeding, solid and sclerosis area). When the lesion is dominated by papillary structures and the other three structures are not obvious, the FS magnify cell atypia and misdiagnosed as invasive adenocarcinoma **[10].** The Vimentin and CK7 antibody could be used in RD-IHC to distinguish the two types of mass. It is difficult to distinguish breast sclerosing adenosis from invasive carcinoma, and there is a certain degree of misdiagnosis rate in X-ray, ultrasound and MRI manifestations **[11,12].** Also, sclerosing adenosis is indistinguishable from carcinoma on microscopy examination. Therefore, pathological diagnosis, especially intraoperative freezing diagnosis, is extremely risky. Most ductal carcinoma in situ may be not difficult to diagnose, but when ductal carcinoma in situ involves sclerosing adenosis with minimal invasion, it presents a great challenge to the pathologist. When the myoepithelial markers, such as P63 and CK5/6, are labeled by RD-IHC, the myoepithelial cells could be clearly displayed and differentiated from invasive cancer easily.

Brain high-grade glioma shows significant nuclear atypia, frequent mitoses, high cell density and obvious microvascular hyperplasia, which is difficult to distinguish with lymphoma morphologically **[13,14].** There is no significant difference between clinical, imaging and laboratory tests. However, the principle of surgical treatment for glioma is maximum safe resection, while surgical resection for lymphoma is controversial. So, intraoperative distinction between high-grade glioma and lymphoma is very important. In this case, RD-IHC staining using GFAP and LCA antibody can serve as an assistant diagnosis of high-grade glioma and lymphoma. Moreover, RD-IHC staining using CK(AE1/AE3) antibody could also be applied inside resection margin or lymph node, which could play guiding role in surgical procedures. The practicability of RD-IHC staining in this study is reflected in two aspects: Firstly, the staining method is very simple and easy. Under normal temperature, it only takes one step (1-3 min) to complete antigen and antibody binding. The total staining time is 8-10 min, which is beneficial for technicians to master the method and has strong operability in practical work. Secondly, the staining effect and area was consistent with routine EnVision method. The intensity of staining is similar with no non-specific immunoreactivity and background reactivity. In this study, antibodies CK(AE1/AE3), CK5/6, P63, P40, GFAP, LCA, syn, Vimentin and CK7 directly labeled with horseradish peroxidase were used for RD-IHC staining in controversial lesions, which significantly improved the intraoperative diagnosis accuracy and reduced delayed diagnosis rate. It could be predicted that more RD-IHC antibodies will be used for c intraoperative pathologic diagnosis of different tissues during surgery. It can have a significant effect on surgical procedures.

In conclusion, rapid intraoperative immunohistochemistry could be a useful tool in intraoperative pathologic diagnosis.

Funding

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