Diagnostic Pathology

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FAP-α is an effective tool to evaluate stroma invasion of lung adenocarcinoma

Siping Xiong[1†], Huan Fan[1†], Yimin Guo[1†], Ruixiang Sun[2], Hongmei Ma[1*], Yali Xiang[1*] and Chao Zeng[1*]

Abstract

The main difficulty in the diagnosis of atypical in situ adenocarcinoma lies in the distinction between true and false stromal invasion. Moreover, how to identify local alveolar wall collapse in situ lung adenocarcinoma and how to identify whether the trapped adenoid structure around scar is an invasion component have become the key points for accurate diagnosis of lung adenocarcinoma. In the present study, we detected 40 cases of lung adenocarcinoma in situ and 40 cases of invasive adenocarcinoma by using immunohistochemical techniques. We found FAP-α had not immunreactivity in the stroma of adenocarcinoma in situ. However, it stained in the stroma of invasive areas in lung adenocarcinoma. FAP-α staining pattern could represent hyperplastic myofibroblast and demonstrated the true invasion of stroma. This study provides strong evidence that FAP-α is an effective tool to evaluate the presence or absence of stroma invasion of lung adenocarcinoma. Our findings will contribute to the accurate diagnosis of lung invasive adenocarcinoma.

Keywords Lung adenocarcinoma, Fibroblast activation protein-α, Smooth muscle antibody, Stroma invasion

Introduction

Lung cancer is still the most common malignancy worldwide. Along with extensive application of immunohistochemical and molecular biology techniques in lung cancer, the study of pathology is progressing rapidly, and new histological types of tumors was discovered and presented with new diagnostic criteria [1–4]. Due to the differences of national conditions, there are some uncertainties in the practical application of some international diagnostic criteria, which often pose challenges to clinicopathological diagnosis.

Adenocarcinoma in situ is the starting point of lung adenocarcinoma. Correct diagnosis of adenocarcinoma in situ is of great significance. However, with the different duration of in situ adenocarcinoma, its tissue structure and cell morphology have varied. Some in situ adenocarcinomas cases had complex tissue structure and florid hyperplasia. The growth of cancer cells are active in some areas and decrease in other areas, which results in the spatial polymorphism of the tissue structure of adenocarcinoma in situ, causing difficulties in diagnosis. The main difficulty in the diagnosis of atypical in situ adenocarcinoma lies in the distinction between true and false stromal invasion. The reduction of cancer cells in alveolar will lead to collapse of the alveolar structure and false stromal invasion. Moreover, the surrounding lung stromal hyperplasia also compress the alveolar structure and form the false stromal invasion. Hence, how to identify

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local alveolar wall collapse in situ lung adenocarcinoma (differentiating it from invasive adenocarcinoma) and how to identify whether the trapped adenoid structure around scar (or consolidation area) is an invasion component have become the key points for accurate diagnosis of lung adenocarcinoma.

Fibroblast activation protein-α (FAP-α) is a serine peptidase that has collagenase /gelatinase activity and participate in the remodeling of the extracellular matrix [5], which is crucial for the disruption of the basal membrane and for the invasion of the intestinal layers by cancer cells [6, 7]. FAP expression has also been associated with the epithelial to mesenchymal transition of cancer cells [8–10]. In the present study, using immunohistochemical techniques, we found that FAP-α is expressed in the mesenchyma surrounding the nests of invasive lung adenocarcinoma, but not in the mesenchyma surrounding in situ adenocarcinoma, suggesting that FAP-α may be an ideal marker for differentiating atypical in situ adenocarcinoma from invasive adenocarcinoma.

Materials and methods

Patients and tissue samples

For immunohistochemistry and elastic fibre dyeing, 40 cases of lung invasive adenocarcinoma and 40 in situ adenocarcinoma were obtained from the Department of Pathology, The Eighth Affifiliated Hospital, Sun Yat-sen University in a period ranging from 2022 to 2023. Males predominated in the series (48 males/32 females), with an average age of 53 years (range: 35 to 70y) for males and 68 years (range: 58 to 72y) for females. All of these histological specimens were reevaluated by two experienced pathologists. This study was approved by the ethics committee of the Eighth Affifiliated Hospital of Sun Yat-Sen University.

Immunohistochemistry

The slides were microwaved in citrate buffer for 8 min for antigen retrieval, and Rabbit anti-human monoclonal FAP-α (1:200 dilution, AB207178, Cambridge, UK) goat anti-human polyclonal SMA (MAB-0890, Maixin, Fuzhou) was applied as the primary antibody. Labeling was detected by horseradish peroxidase-conjugated mouse anti-goat IgG and staining with 3,3′-diaminobenzidine (Maxim-Bio, Inc., Fuzhou, China). Finally, the slides were counterstained with hematoxylin.

Elastic fibre dyeing

The slices were washed in 70% ethanol for 2 min and dyed in Victoria. Blue solution at 37℃ overnight. Anhydrous ethanol washed excess dye. Lastly, the slices were sealed with neutral gum.

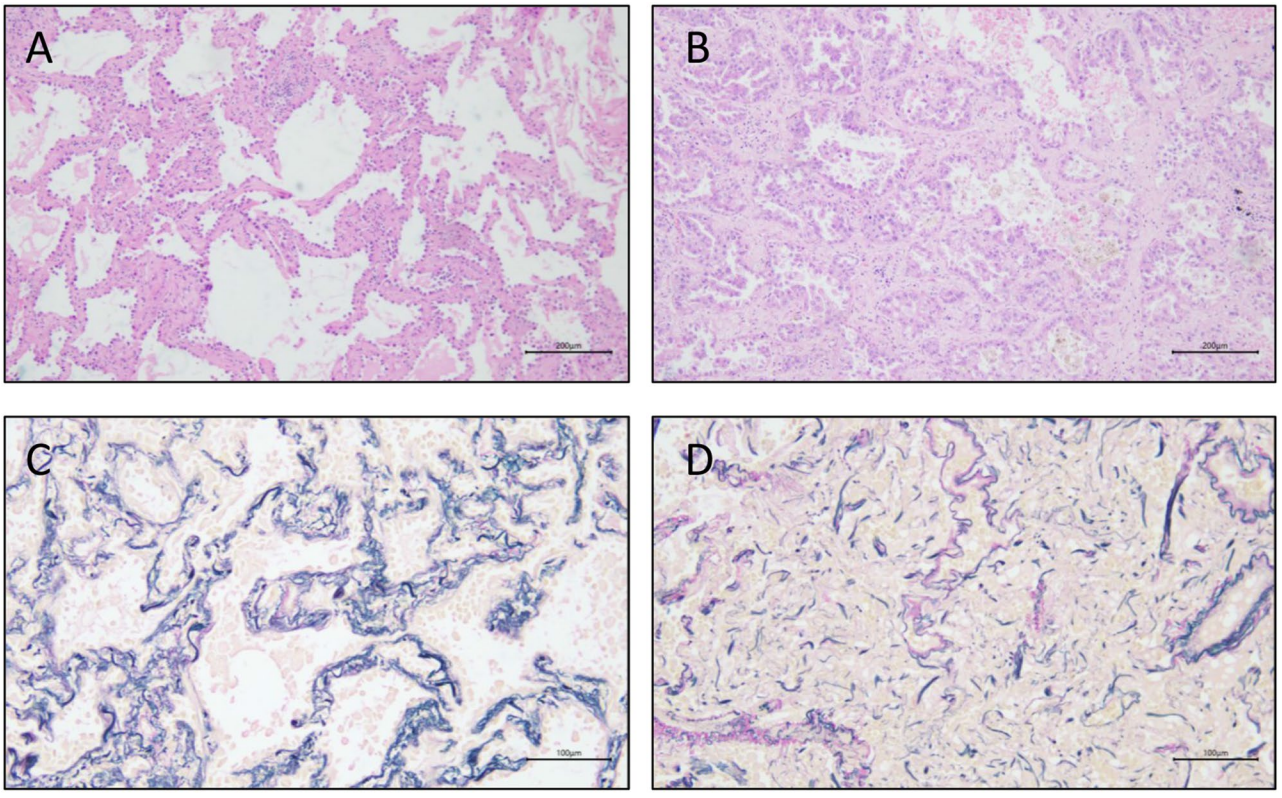

Fig. 1 HE& Elastic fibre dyeing. The representative image of in situ adenocarcinoma (**A**) and invasive adenocarcinoma (**B**). The representative elastic fibre dyeing image of in situ adenocarcinoma (**C**) and invasive adenocarcinoma (**D**)

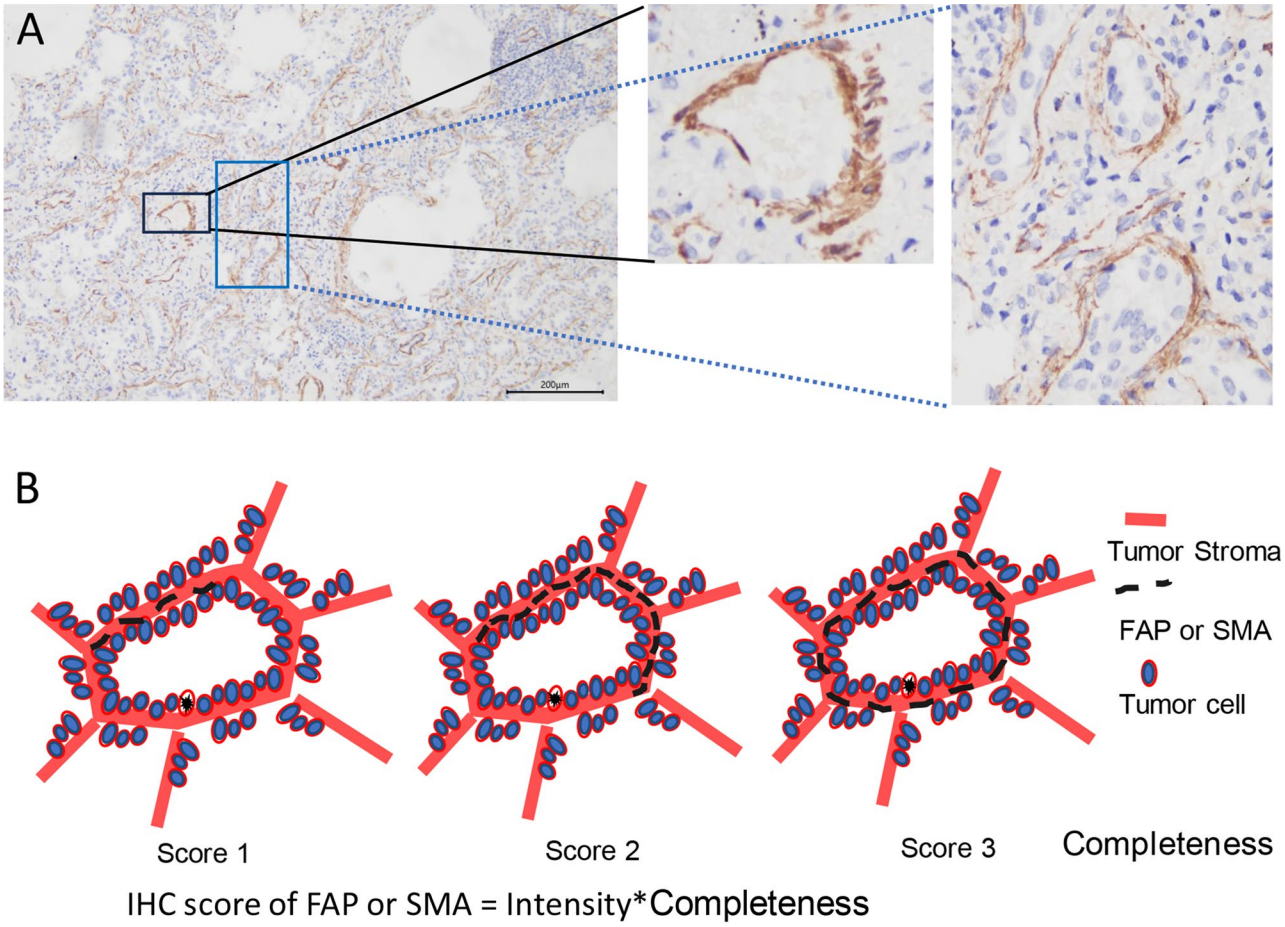

Fig. 2 Evaluation of IHC score. (**A**) Both smooth muscle cell (black box) and myofibroblast cell (blue box) are stained by SMA antibody. (**B**) Schematic graph to evaluate IHC score of SMA or FAP

Statistical analysis

Statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). The $\chi 2$ test was used to analyze the categorical expression of FAP-α and SMA in invasive lung adenocarcinoma and in situ adenocarcinoma. Receiver operating characteristic curves were produced to determine the sensitivity, specifificity, and area under the curve.

Result

HE& elastic fibre dyeing

Lung invasive adenocarcinoma and in situ adenocarcinoma were diagnosed by 3 pathologists. The representative images were displayed in Fig. 1A-B. However, the Elastic fibre dyeing was hard to evaluated. Only several cases could be confirmed by Elastic fibre dyeing. The typical photo of in situ adenocarcinoma was showed in Fig. 1C, while the invasive adenocarcinoma was displayed in Fig. 1D.

Approach to calculate immunohistochemistry (IHC) score

To better evaluate the expression of SMA and FAP-α, a novel method was applied. For SMA, the true signal should be identified. As shown in Fig. 2A, the black box displayed that smooth muscle cells of vessel were also stained, while the blue box indicated the true signal of invasive stroma. Next the IHC score was calculated by multiplying intensity by completeness (Fig. 2B). Less than 1/3 completeness got 1- point, 1/3 to 2/3 completeness got 2-points, more than 2/3 completeness got 3-points. Weak intensity got 1-point, medium intensity got 2-points, strong intensity got 3- points. The total score was equal to intensity multiplied by completeness.

Expression level of SMA

The expression level of SMA was evaluated with the above novel method. The expression level of SMA in situ adenocarcinoma was low, most of them are negative (Fig. 3A). While in the invasive adenocarcinoma, the expression level of SMA was high (Fig. 3B). The results displayed that the IHC score of SMA in invasive adenocarcinoma was significantly higher than that in in-situ

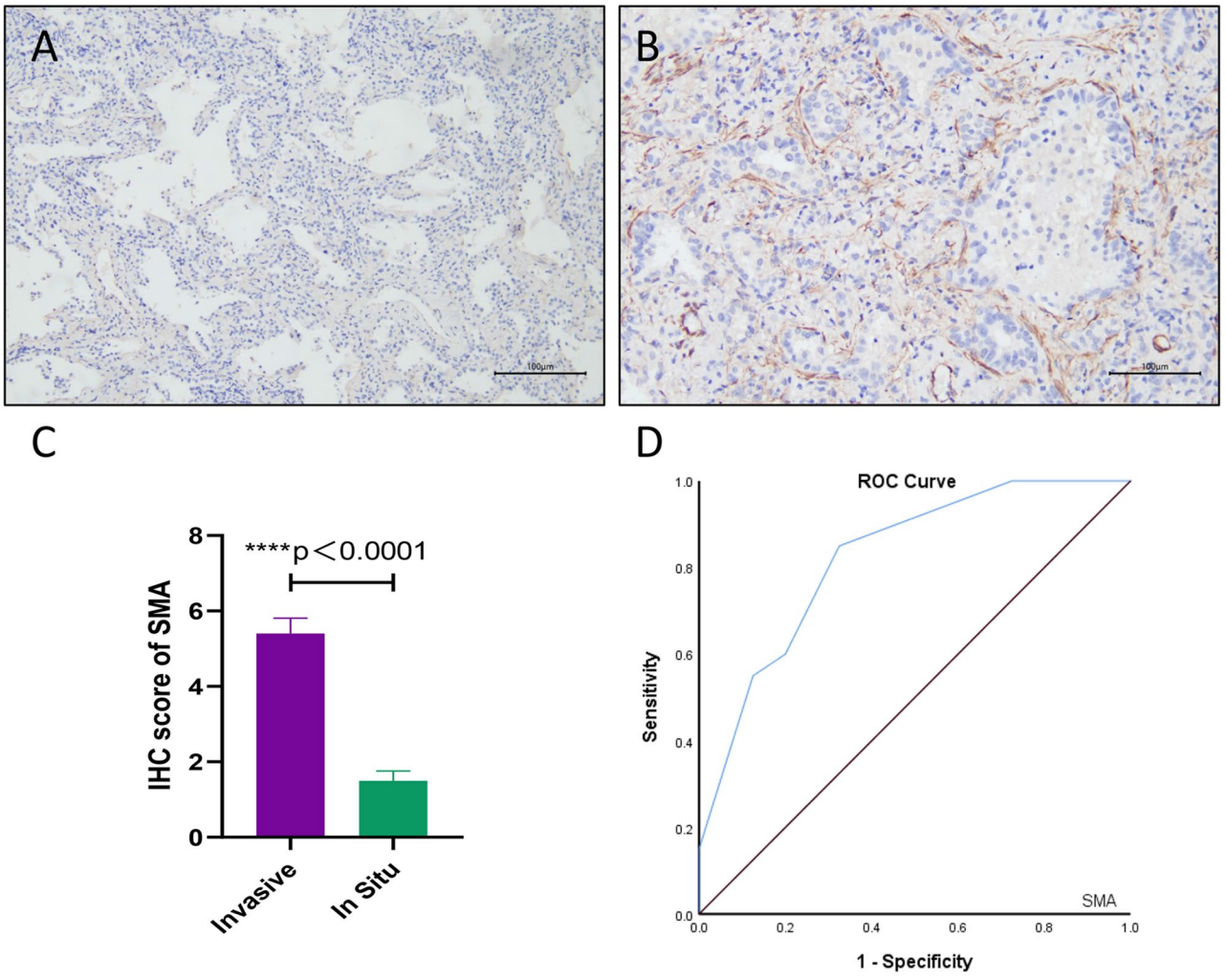

Fig. 3 SMA expression. The representative SMA immunohistochemistry image of in situ adenocarcinoma (**A**) and invasive adenocarcinoma (**B**). IHC score of SMA (**C**). ROC curve of IHC score (**D**)

adenocarcinoma (Fig. 3C). According to the IHC score, we got a Receiver Operating Characteristic (ROC) curve with an AUC (the area under the ROC curve) of 0.816 as shown in Fig. 3D. The cut-off value of SMA-IHC score was 2 points.

Expression level of FAP-α

The expression level of FAP-α was also evaluated with the above novel method. With the similar results of SMA, the representative images were exhibited as Fig. 4A-B. Further, the results displayed that the IHC score of FAP-α in invasive adenocarcinoma was significantly higher than that in in-situ adenocarcinoma (Fig. 4C). According to the IHC score, we got a Receiver Operating Characteristic (ROC) curve with an AUC (the area under the ROC curve) of 0.850 as shown in Fig. 4D. The cut-off value of FAP-α -IHC score was 4 points.

Comparison of FAP with SMA

According to the cut-off values, 34 cases were classified as SMA-positive among 40 cases of invasive adenocarcinoma. And 27 cases were classified as SMA-negative among 40 cases of in situ adenocarcinoma (Fig. 5A). However, 29 cases were classified as FAP-α-positive among 40 cases of invasive adenocarcinoma. And 37 cases were classified as FAP-α-negative among 40 cases of in situ adenocarcinoma (Fig. 5B). Even if, the specificity and sensitivity were not statistically significant between SMA and FAP-α (Fig. 5C). The FAP-α (0.925) had better specificity than SMA (0.675), while SMA (0.85) had better sensitivity than FAP-α (0.725).

Discussion

The presence or absence of stromal infiltration is a key step in the diagnosis of lung adenocarcinoma, because it is related to the surgical method and the patient's prognosis. In most cases, it is easy for pathologists to make

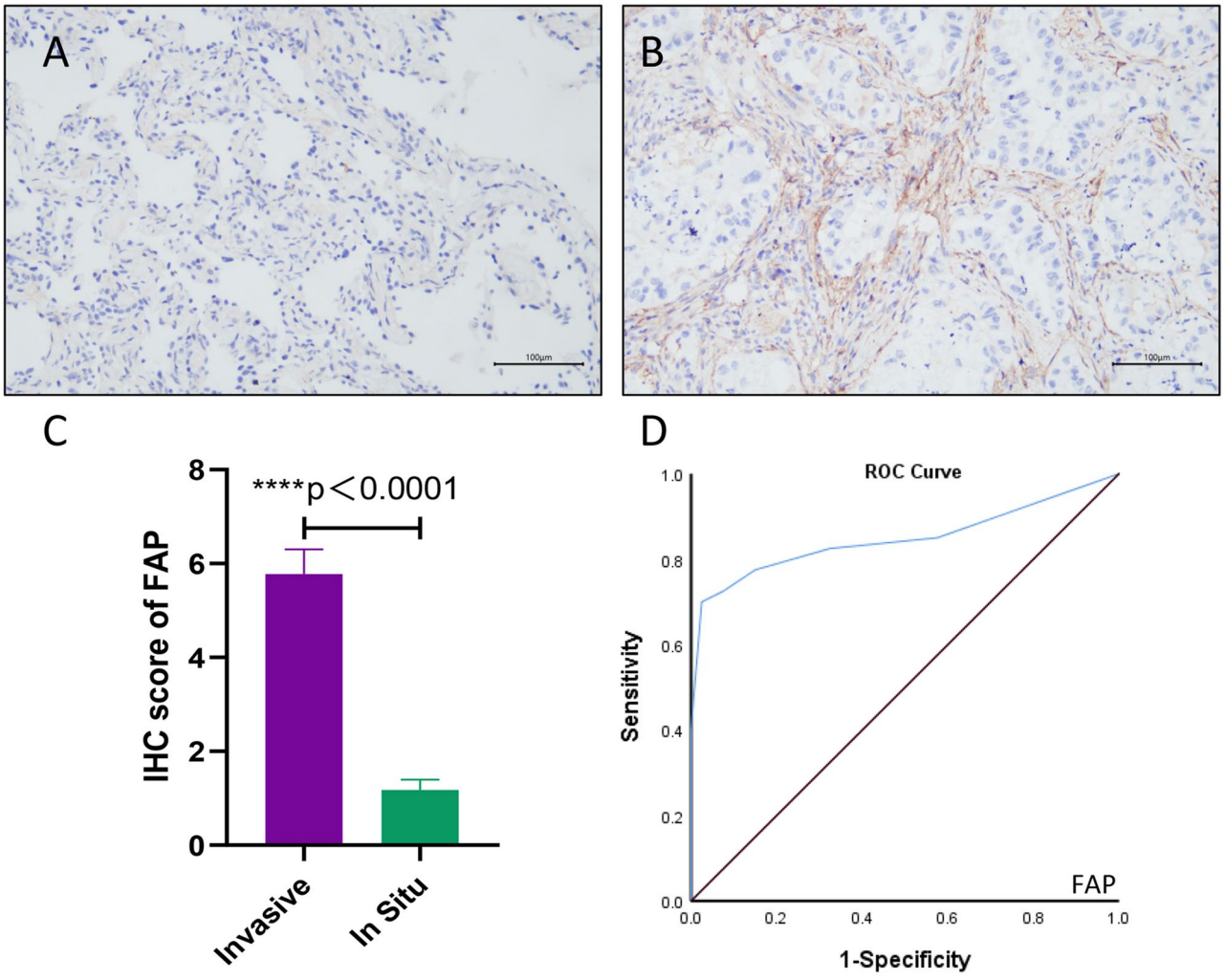

Fig. 4 FAP expression. The representative FAP immunohistochemistry image of in situ adenocarcinoma (**A**) and invasive adenocarcinoma (**B**). IHC score of FAP (**C**). ROC curve of IHC score (**D**)

A

SMA	Diagnosis	
	Invasive	In-situ
Positive	34	13
Negative	6	27

B

FAP	Diagnosis	
	Invasive	In-situ
Positive	29	3
Negative	11	37

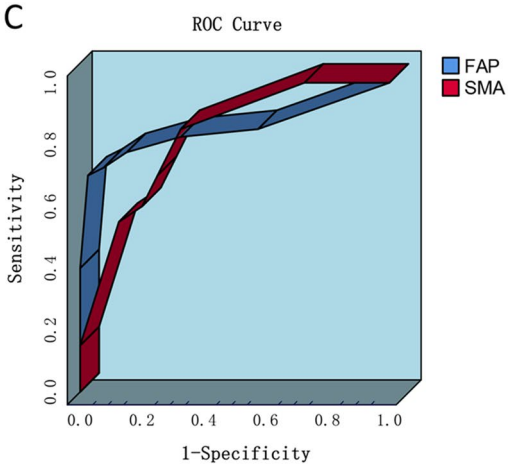

Fig. 5 Comparison of SMA and FAP. (**A**) The correlation of SMA with invasion. (**B**) The correlation of FAP with invasion. (**C**) Comparison of sensitivity and specificity between FAP and SMA

a diagnosis of invasive adenocarcinoma. However, in a few cases, the widening, sclerosis and collapse of alveolar septum can easily lead to the misdiagnosis of the lepidic structure as acinar adenocarcinoma. The core of differentiation is to determine whether the stroma is the original alveolar septum. When myofibroblastic hyperplasia occurs in the tumor stroma, it should be identified as true acinus.

Lung adenocarcinoma is a multifaceted disease with diversity in histology, including micropapillary pattern, papillary pattern, acinar pattern, lepidic pattern and solid pattern [11–14]. It is relatively easy to distinguish between micropapillary, papillary and solid types. Difficulties in distinguishing acinar pattern from lepidic pattern are not so obvious, especially in cases of lung adenocarcinoma with widening of alveolar septum and collapse of alveolar cavity. Florid hyperplasia of adenocarcinomas in situ grows along alveolar walls will mimic papillary structures and desmoplastic reaction will produce acinar structures which in reality are collapsed areas lacking invasion. In order to better identify true invasion and false invasion, we evaluated the FAP-α staining pattern in lung adenocarcinoma.

Unexpectedly, in this study, we found FAP-α had not immunreactivity in the stroma of adenocarcinoma in situ. However, it stained in the stroma of invasive areas in lung adenocarcinoma. FAP-α staining pattern could represent hyperplastic myofibroblast and demonstrated the true invasion of stroma. Interestingly, Alexandre reported that FAP-α identifies stromal invasion in colorectal adenocarcinoma [15]. FAP is involved in extracellular matrix remodeling with collagen being a key substrate, thereby facilitating tumor migration [16]. These findings revealed that FAP-α has a unique role in identification of cancer invasion. In the present study, the role of SMA and elastic fiber staining in the diagnosis of lung invasive adenocarcinoma was also investigated. Although the role of SMA in identifying invasive lung adenocarcinoma is similar to that of FAP-α, the specificity of SMA in judging stroma invasion was lower than that of FAP-α. Our results indicated that SMA could stain not only myofibroblasts but also vascular smooth muscle and perivascular cells. Compared with adenocarcinoma in situ, the distribution of elastic fibers in the invasive adenocarcinoma area is more disordered. The defects of elastic fiber dyeing are mainly affected by the subjective judgment of the evaluator. Therefore, FAP-α is a better indicator than SMA and elastic fiber staining in judging lung invasive adenocarcinoma. We speculate that FAP may also play an important role in evaluating the stromal invasion of other digestive system tumors (gastric cancer, esophageal cancer, etc.).

In summary, given that some cases of lung adenocarcinoma show difficulty in judgment of stroma invasion, this study provides strong evidence that FAP-α is an effective tool to evaluate the presence or absence of stroma invasion of lung adenocarcinoma. Our findings will contribute to the accurate diagnosis of lung invasive adenocarcinoma. Furthermore, our findings will also provide experimental basis for clinical treatment planning of lung adenocarcinoma and evaluation of prognosis.

Author contributions

Siping Xiong and Yimin Guo wrote the main manuscript text. Huan Fan finished the experimens. Ruixiang Sun analysis the data. Hongmei Ma wrote and reviewed the main manuscript text. Yali Xiang wrote and validated the main manuscript text. Chao Zeng wrote and validated the main manuscript text.

Funding

This project was partially funded by a grant from Shenzhen Science and Technology Program (JCYJ20220530144407017).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

This study was approved by the ethics committee of the Eighth Affifiliated Hospital of Sun Yat-Sen University and obtained informed consent from the study patients.

Competing interests

The authors declare no competing interests.

Received: 24 May 2024 / Accepted: 13 November 2024
Published online: 25 November 2024

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