In this manuscript, the authors characterize the expression of selected protein markers in lung fibroblasts in different types of lung diseases, including #SMA, FAP, Vimentin, E-cadherin, cytokeratin-19 (CK-19), TGF-# and twist. Utilizing Immunohistochemistry (IHC) on tissue sections of human specimens ranging from normal lung to node positive adenocarcinoma, the authors show a correlation between the expression of #SMA, Vimentin and CK-19 and the degree of the malignancy, while E-Cadherin shows an inverse trend. This gradual activation of Fibroblasts was evident only within the cancerous tissue but not in adjacent normal lung tissue.

The results of this study, while not completely novel, are relevant and mostly of high quality and support the known role of the tumor microenvironment, CAFs in particular, in tumor progression and metastasis, suggesting that CAFs may be an attractive target for adjuvant therapies.

The manuscript contains human data with a relatively large number of patients, which is one of its strengths. However, some of the results are presented in a confusing manner and the terminology used is inaccurate.

Specific comments:

1. The presented IHC and figures are of high quality and convincing. However, it would be useful to add arrows to mark the relevant cells in each figure.

2. Throughout the manuscript, the authors use the term “CAFs” repeatedly to describe activated fibroblasts in inflammatory diseases or in healthy tissue. Per definition, CAFs are Cancer-Associated and do not exist elsewhere.

3. In the methods section the authors describe the IHC analysis: “200 cells were scored” – it is unclear weather 200 fibroblasts were scored and if so, how were they recognized. The fact that 200 fibroblasts were counted per field in all tissue types seems unlikely. Since this staining is central to the presented work work, a more detailed description of the analysis methods is required.

4. The first paragraph in the results section describes the staining of #SMA and Vimentin in different stages. The text goes back and forth between #SMA and Vimentin, in a repetitive and confusing manner. A more organized description of the results, preferably describing each protein would make this paragraph more understandable.
5. This same paragraph (p. 4) contains an internal contradiction: “#-SMA was detected in benign and malignant tissues and its expression was localized to blood vessels and airway ducts in normal lung tissues and atypical adenomatous hyperplasia” and “#-SMA and Vimentin showed positive expression in stromal cells of benign lung tissue (I) and SI, similar to their expression in CIS”.

So which is it? Is #-SMA expressed only around blood vessels and airway ducts in benign lung tissues or was it positive?

6. The abbreviation “SI” is used in this paragraph for the first time and is not explained in the text or in the list of abbreviations.

7. In section 3.2.2. of the results, FAP expression is analyzed along with TGF-# and twist. FAP was previously described as a marker of both normal fibroblasts and CAFs in other organs and malignancies, but not in cancer cells. This is also stated several times by the authors. Since the data presented here does not agree with previous publications, they should be discussed in more detail in the discussion, in order to put these results in the context of the field.

8. In the discussion section (bottom of p.7) the authors state: “our results showed that FAP was expressed in the stroma of cancer cells rather than in CAFs”. This sentence does not make sense, as CAFs are an integral part of the cancer stroma. Please clarify.

9. There have been several recent studies describing CAFs in lung carcinoma. They should be sited. (Examples:

   “Transforming growth factor-#1 and #-smooth muscle actin in stromal fibroblasts are associated with a poor prognosis in patients with clinical stage I-IIIA nonsmall cell lung cancer after curative resection”. Chen Y. et al. 2014.


Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.