REPORT FOR AUTHORS

In this manuscript entitled “Fibroblast Phenotypes in Different Lung Malignancies” authors presented an attempt to characterize stromal cells in several lung malignancies by immunohistochemical analysis of patient samples obtained during surgery. Although the manuscript presented the results of a fair large number of patients, there are several points that were not very clear and need to be addressed before presenting to the scientific community:

Major Compulsory Revisions

1) The manuscript requires “substantial” English language editing.

2) Page 1, Line 3: “The role of CAF in lung cancer has been previously investigated”, This statement is not supported in the main text, there is no reference. Also if the role has been previously studied, please provide the main findings.

3) Page 1, Line 4: “CAFs may also play a role in inflammatory disease”, here there is a fundamental misunderstanding of the term CAF by the authors. CAF as they stated stands for “CARCINOMA associated fibroblasts” emphasizing the presence “only” in the microenvironment of malignant disease. Reactive stroma, activated fibroblasts or myofibroblasts can be seen in inflammatory diseases and in wounds during the process of repair. Please provide a better explanation of the term or use an alternative.

4) Page 1 second line in the Background section: What does “bad” cancer cells mean? Cancer cells are malignant in nature, perhaps the authors meant cancer cells that will show progression vs. those that will remain localized? Please explain.

5) Reference 3 by Xing et al is a review in which the authors describe the characteristics of CAF (expression of #-SMA, vimentin,FAP and other markers) and lack of CD31 and epithelial markers without mentioning CK19 loss. Please provide a reference of the lack of CK19 expression by CAF.

6) Results, 3.2.1 Detection of CAFs and epithelial markers: In the first sentence the authors state that #-SMA is expressed in blood vessels and airway ducts in normal and in H (atypical adenomatous hyperplasia) tissues, however 6 lines below the state” N and H tissues were negative for #–SMA expression (?), Please explain. In figure 1 #–SMA can be seen expressed in all groups with the highest percentage (optical) and intensity in I>AM>CIS>A>H>N
7) The differences between the groups should be presented with a graphical representation using bars or dot plot along with IHC images to see a better stratification of patients within each group.

8) Quantitation of the intensity is a somewhat subjective assessment. There are several digital methods published in the scientific literature that can be used to validate the results. If the authors have access to frozen tissues a quantitative measure by qRT-PCR or protein analysis can be more informative.

9) Figure 1, vimentin expression, same concerns as αSMA

10) Cytokeratin 19 expression in Figure 2; poor tissue in CIS sample, please use consecutive slices of tissues.

11) Figure 3. TGFβ: assessment of TGFβ expression by IHC is very difficult. This secreted protein can be present in the epithelial and stromal compartment, perhaps staining with phosphor-Smad2/3 can show activation of the TGFβ pathway in tumor or stromal cells. Again if frozen tissues are available it can be coupled to the total expression of TGFβ by the tissue, but it cannot identify the cell secreting the protein. Sorting cells by flow cytometry (epithelial vs stromal cells) will answer this question.

12) It will be useful to have the N, I and H groups in Fig3 to compare the level of TGFβ, Twist and FAP with the other groups

13) Twist expression seems to be present in the nucleus. Authors claim to be in the cytoplasm. Explain

14) FAP is considered a stromal marker (Pubmed; PMID: 23835897), however the authors found localization in cancer cells with increased expression in CIS compared to the other groups based on the IHC images. Was the quantitation made considering only stromal FAP?

15) The A group show “nuclear” FAP? Authors used a rabbit antibody purchased from SantaCruz Biotechnology. Santacruz sells five antibodies against FAP (three goat, one mouse and one rabbit), none of them are recommended for IHC. The rabbit antibody FAP# (clone H-56) is a protease that can be found in the cytoplasm or ECM. Can the authors provide validation of this antibody for IHC?

16) Several papers have addressed the complex nature of the tumor stroma. Due to the high heterogeneity and the lack of a “single” marker of CAF, attempts have been made to show co-expression at the cellular level of these potential markers. Thus double/triple co-expression of the most abundant primers and categorizing with each group should be presented.

17) Figure 4. This graphs are rather confusing. Were the comparisons made for each group or all the gourps combined? What do the individual circles represent (patient/s)? Why are there different number of circles in each graph? Are the X and Y axis representing SI index? If so why the scale is different in some, instead of 10 scale (first graph) up to 4 or 6 in the others? Please show consistency and better labeling. Perhaps a better representation would be to show a table and correlations.

18) The major question(s) of the paper has not been answered. Do the CAF
phenotype vary in lung disease, if so, can this variation explain tumor progression?

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I do not have any particular interest