Response to reviewers

We appreciate the reviewers for their important comments which improve our manuscript greatly.

Reviewer #1:

Jang and colleagues applied the CanPatrol technique coupled to RNA-ISH to classify CTCs using EMT markers in patients with lung cancer. Compared with healthy controls and benign tumors, CTCs with mesenchymal features were more commonly found in patients in the advanced stages of the disease, showing acceptable specificity and sensitivity of the method. The work is interesting and adequately presented, adding new clues to the possibility for implementing the CanPatrol protocol in early diagnosis of lung cancer. As the authors mentioned, the study will necessitate of further validation in bigger and prospective cohorts.

Response: We totally agree with the reviewer. A well designed multi-center study with robust setup of cases and controls are necessary for further validation.

Reviewer #2:

This is an interesting, well written paper that suggests a role for detecting circulating tumor cells (CTCs) in diagnosing lung adenocarcinomas. While, as the authors indicated, further studies will be necessary to confirm how significant this finding will be as a front-line diagnostic tool, the results are encouraging. A few questions remain.

1）It was not clear how any of the tissue and blood sampling was "blinded" as to its derivation, and how such labeling could play any role in biasing the results.

Response: The blood samples were sent to the lab for CTCs detection without the detailed information of subjects. Blood samples were detected in accordance with the standard procedures. Also, the staff members of the lab did not know final pathological diagnosis of these subjects. We strictly followed the inclusion and exclusion criteria, and all data were processed by two people separately. All above mentioned measures were taken to reduce bias.

2）The data show that "benign" tumors were a source of CTCs and the authors indicated that such cells "can be shed" and thus collected and analyzed. Is anything known about the nature of these "benign" tumors such that perhaps they were not as "benign" as originally described, or that the tumors were perhaps in a pre-malignant stage?

Response: It is a very important question. In our article, benign tumors were confirmed by the postoperative pathological examination. Systemic physical and radiological examination exclude the occult cancer.

As we mentioned in the revised discussion(Page 10), CTCs can be recognized as a “sentinel” in cases associated with inflammatory diseases such as COPD[1]. similarly, in patients with benign colon diseases, CTCs can also be detected with both the CellSearch system (11.3%) and the CK19-EPISPOT assay (18.9%), because inflammatory epithelial cells from these benign lesions may enter the peripheral blood[2]. In 232 patients with benign breast diseases, the CTC detection rate reached 15.95%[3], Herein, we hypothesized that tumor cells or debris, which can be shed from the benign tumor into the peripheral blood, are captured and recognized as CTCs by CanPatrol ™.

Indeed, we agreed with the reviewer that perhaps these "benign" tumors or diseases were in a premalignant stage, because CTCs may appear at a very early stage of cancer development, even if the lesion is undetectable by systemic physical and radiological examinations. However, this inference requires long-term follow-up.

We add this part into revised discussion (paragraph6, page10-11).

3) Ground glass opacities (GGO) are a common finding in lungs with scarring and ongoing fibrogenesis. Since the lung cancers in concert with GGO in this study were likely to yield CTCs, a question could be asked about the degree of ongoing fibrogenesis and how its severity might have correlated with the finding of CTCs. Since many tumors are found in a background of tumor- induced fibrosis, a question could be asked to whether the fibrosis was due to the tumor or perhaps by the original carcinogens and how this point might correlate with the CTC findings.

Response: It is an important point. we totally agree with the reviewer that GGO are a common finding in lungs with scarring and ongoing fibrogenesis. Intriguingly, fibrosis can be found in either benign or malignant lung GGO[4, 5]. However, very few published studies focused on CTCs and GGO, not to mention CTCs and fibrosis.

Furthermore, pathological report from our hospital did not clearly describe the fibrosis condition of these GGO cases. Therefore, we are going to design further clinical trials regarding CTCs in GGO, which will consider the fibrosis condition and the expressions of relevant biomarkers, e.g., CXCL4 and LECT2[6, 7].

We added the abovementioned part into revised discussion (paragraph8, page11).

Reviewer #3:

The article "Circulating tumor cells with epithelial-mesenchymal transition markers as potential biomarkers for the diagnosis of lung cancer" is an interesting article. The statistics in the figure should be drawn separately for both positive vs control, and negative vs control. The discussion and the flow of story should be more concise and clear.

Response: As per the reviewer, we have changed the Figure 1B into positive rate, which will be more clear. The discussion and the flow of story had been revised by International Science Editing (http://www.internationalscienceediting.com).

[1] Ilie M, Hofman V, Long-Mira E, Selva E, Vignaud JM, Padovani B, et al. "Sentinel" circulating tumor cells allow early diagnosis of lung cancer in patients with chronic obstructive pulmonary disease. PloS one 2014;9:e111597.[PMID:25360587; DOI:10.1371/journal.pone.0111597]

[2] Pantel K, Denève E, Nocca D, Coffy A, Vendrell JP, Maudelonde T, et al. Circulating epithelial cells in patients with benign colon diseases. Clinical chemistry 2012;58:936-40.[PMID:22205690; DOI:10.1373/clinchem.2011.175570]

[3] Jin L, Zhao W, Zhang J, Chen W, Xie T, Wang L, et al. Evaluation of the diagnostic value of circulating tumor cells with CytoSorter(®) CTC capture system in patients with breast cancer. Cancer medicine 2020;9:1638-47.[PMID:31908156; DOI:10.1002/cam4.2825]

[4] Qin Y, Xu Y, Ma D, Tian Z, Huang C, Zhou X, et al. Clinical characteristics of resected solitary ground-glass opacities: Comparison between benign and malignant nodules. 2020;11:2767-74.[PMID:32844603; DOI:10.1111/1759-7714.13575]

[5] Lv YG, Bao JH, Xu DU, Yan QH, Li YJ, Yuan DL, et al. Characteristic analysis of pulmonary ground-glass lesions with the help of 64-slice CT technology. European review for medical and pharmacological sciences 2017;21:3212-7.[PMID:28770963]

[6] van Bon L, Affandi AJ, Broen J, Christmann RB, Marijnissen RJ, Stawski L, et al. Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. The New England journal of medicine 2014;370:433-43.[PMID:24350901; DOI:10.1056/NEJMoa1114576]

[7] Xu M, Xu HH, Lin Y, Sun X, Wang LJ, Fang ZP, et al. LECT2, a Ligand for Tie1, Plays a Crucial Role in Liver Fibrogenesis. Cell 2019;178:1478-92.e20.[PMID:31474362; DOI:10.1016/j.cell.2019.07.021]