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Dear Patporn Chankong:

We would like to thank for your letter and for the reviewer's comments concerning our manuscript entitled: **" Role of ferroptosis in regulating the epithelial–mesenchymal transformation in pulmonary fibrosis" (ID: biomedicines-2078725).** Those comments are all valuable and very helpful for revising and improving our paper, as well as have an important guiding significance for our researches. We have studied comments carefully and have made corrections which hope meet with approval. Revised portions are marked up using the "Track Changes" function in the paper. The main corrections in the paper and the responds to the reviewer's comments are as following:

1. Authors need to confirm that they have the right name of the process they are reviewing here. Should EMT be epithelial-mesenchymal 'transition' or 'transformation'? They use 'transition' in the abstract and then use 'transformation' in the remainder of the manuscript.

Response to reviewer's comment: It is a good reminding. Transition should be used in this paper because transition can express the essential changes from epithelial cells to mesenchymal cells, while transformation cannot.

2. I'm not sure ferroptosis can still be defined as new, researchers have been publishing on it for 10 years now.

Response to reviewer's comment: Thank you for your comment. Ferroptosis is

indeed not a newly discovered method of cell death. The expression in the first sentence of the second paragraph of Introduction is inaccurate and the "new" has been removed. But ferroptosis regulation of EMT to prevent pulmonary fibrosis is a new idea.

Ferroptosis is a ~~newly characterized~~n iron-dependent non-apoptotic regulated cell death[12, 13]. The accumulation of excessive intracellular iron, depletion of glutathione (GSH), inactivation of glutathione peroxidase 4 (GPX4), and upregulation of lipid peroxi-

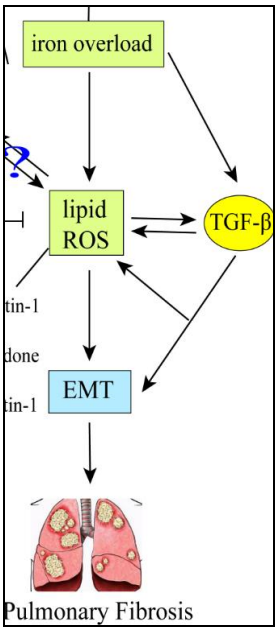
3. There are a lot of grammatical errors in the manuscript, as well as repetition which makes the work difficult to read e.g lines 59-71 has grammatical errors, words missing and also needs more details (such as how do the ferroptosis markers listed have a strong regulatory effect on EMT?).

Response to reviewer's comment: Thank you for your comment. I have carefully revised the inadequate parts of the text, and the manuscript had been read and corrected by inviting professional English editors of LetPub before submission of the revised manuscript.

Line 60 refers to three ferroptosis markers in which GPX4 and CAV1 are repeated in the later text and express the meaning that ferroptosis regulates EMT markers. It is therefore more coherent to change the paragraph to the latter. See the red section in lines 193-196. Screenshot is as follows:

Yao *et al.*[64] found that some ferroptosis markers (GPX4, SCP2, and CAV1) have strong regulatory effects on EMT. SCP2 is a driver of ferroptosis, promoting other ferroptosis drivers (PRKAA1, PRKAA2) and EMT markers (N-Cadherin), but inhibiting ferroptosis inhibitors such as GPX4 and CAV1. Caveolin-1 (Cav-1) is an integral membrane protein involved in cell signal transduction and transport. It is highly expressed in adipocytes, endothelial cells, fibroblasts and cancer cells[65]. Knockdown of Cav-1 can reduce the expression of GPX4, and it is negatively correlated with ROS. Cav-1 is a negative regulator of TGF-β1 activity by reducing the subsequent effects of TGF-β1, leading to a decrease in collagen type I and fibronectin and an increase in MMP mRNA expression[66]. Cav1-deficient peritoneal mesothelial cells acquire the ability to degrade ECM, which is character-

lines 59-71 are analyzed according to part of contents in Figure 4, which are not clearly expressed and have been modified.



in lung tissue sections[23]. Meanwhile, iron metabolism may regulate transforming growth factor-β (TGF-β)-induced EMT through ROS production in the alveolar epithelium ~~or directly induce EMT by ROS~~, then pulmonary fibrosis occurs[24]. Furthermore, *in vivo* TGF-β1 induces ROS production in epithelial cells and inhibits antioxidant enzymes, leading to redox imbalance. ROS, in turn, induces or activates TGF-β1 and causes pulmonary fibrosis, ~~TGF-β-induced EMT and pulmonary fibrosis, in the process of TGF-β-induced EMT, there is an imbalance between oxidation and cellular iron homeostasis, promoting the production of ROS. ROS further induces EMT~~, creating a vicious cycle [25]. Understanding the role of ferroptosis in pulmonary fibrosis may provide a novel therapeutic direction for the prevention and treatment of this disease.

4. Authors write about ROS production and that this leads to ferroptosis several times in the manuscript, however ROS production can lead to several different cell death pathways, and authors don't make enough of a distinction between how or why ROS specifically leads to ferroptosis and not other cell death pathways in pulmonary fibrosis.

Response to reviewer's comment: Thank you for your comment. Not all ROS are associated with ferroptosis. The ROS mentioned in the article is the ROS produced by the reduction of GSH after lipid peroxidation, which will result in ferroptosis. This part is not described and has been added to the Discovery of ferroptosis.

5. Several paragraphs have statements/several sentences that don't fit what the paragraph is describing or are not relevant to the topic being talked about.

o E. g. "iron cells that transport oxygen become prone to death or die" line 113 doesn't follow on from the previous statements in the paragraph.

Response to reviewer's comment: Thank you for your comment. The sentence has been deleted and other inconsistent parts of the article have been revised.

6. Figures: need some work to show what the authors are describing (and legends describing what the figure shows with the abbreviations explained).

o Figure 1 is titled "Ferroptosis regulates EMT in pulmonary fibrosis via the TGF-b/Smad signalling pathway" however, the figure doesn't show this. The figure shows what role iron may play in the signalling pathway, but not ferroptosis. The pathway can not be called ferroptosis if you don't show a link to how this induces lipid peroxidation and subsequent cell death.

§ Liproxstatin-1 (spelled incorrectly in the text)

§ Figure should also show how the expression of Smad leads to EMT.

o Figure 2 – also has some missing elements:

§ Again the same point about the figure not showing ferroptosis regulating EMT, but how the separate elements of iron overload and oxidative damage may interact. How does lipid peroxidation fit into this?

§ Where is the oxidative damage coming from?

§ The red dots in the figure make the text unclear ('nucleus' is not very clear).

Response to reviewer's comment: Thank you for your comment. Figure legends are behind the references and has been modified according to the figure. Figure 1 has now shown that ROS causes ferroptosis after lipid peroxidation, and it is clear that it will eventually produce ferroptosis. The process of Smad leading to EMT was added, and Liproxstatin-1 was modified. In Figure 2, oxidative damage caused by oxidative stress caused by iron accumulation in epithelial cells has been identified, and the nuclear picture has been lightened to make the text clear.

Figure Legends

Figure 1. Ferroptosis regulate EMT in pulmonary fibrosis via the TGF-β/Smad signaling pathway. TGF-β non-covalently binds to latency-associated peptide (LAP) to form an small latent complex (SLC) that blocks TGF-β from binding to its receptor. The SLC connects with LTBP in the endoplasmic reticulum to form the large latent complex (LLC). After TGF-β release by LAP, TGF-β binds to TGFβR-II to phosphorylate TGFβR-II, activates TGFβR-I, and phosphorylates intracellular Smad2 and Smad3. Phosphorylated Smad2 and Smad3 interact with Smad4 and translocate to the nucleus and regulate target gene transcription by binding to DNA-binding transcription factors. The ferroptosis activator RSL3 can inhibit GPX4, thereby increasing ROS, promoting ferroptosis and idiopathic pulmonary fibrosis. Ferroptosis inhibitors liproxstatin-1 inhibit ROS and have the opposite effect. Meanwhile, TGF-β enhances ZEB1-mediated EMT to promote pulmonary fibrosis. ACO1/IRP1 plays a role in regulating intracellular iron homeostasis. Cav-1 attenuates Smad3 nuclear translocation, and the NDRG1 gene inhibits pulmonary fibrosis by inhibiting the Smad2/3 phosphorylation gene. Smads gene promotes the expression of N-cadherin and Vimentin, and inhibits E-cadherin, thus promoting EMT.

Figure 2. Ferroptosis regulate EMT in pulmonary fibrosis via the Nrf2 signaling pathway. Under normal conditions, Keap1 binds to Nrf2, which is degraded by ubiquitination. However, when iron accumulates in epithelial cells, the imbalance between oxidation and antioxidant reaction results in oxidative stress. Nrf2 dissociates from Keap1 during oxidative stress, and Nrf2 enters the nucleus to form heterodimerization with small Maf or Jun proteins. This dimer binds to ARE transcriptional activate its target genes and restore cell homeostasis. Bach1 regulates EMT through CLDN3 and CLDN4, and Bach1 regulates FOXA1 and snail2, forming GRN to regulate EMT. Bach1 decreased the expression of the E-cadherin gene and promoted the occurrence of EMT. Meanwhile, Bach1 directly activates snail2, which promotes EMT by inhibiting cell-cell adhesion. Knockdown of Nrf2 significantly reduces heme oxygenase 1 (HO-1), promotes the accumulation of lipid peroxidation, and causes ferroptosis.

We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions. If you have any queries, please don't hesitate to contact us.

Yours sincerely,

Jiang Deng Prof. Jiang Deng