Peer Review File

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Reviewer A

Liu et al. investigates the role of CFTR in B-ALL, which bases on their previous work about CFTR. The authors uses a cohort of human childhood patients and found that CFTR expression is increased both at protein and mRNA level. Using immunofluorescence the authors show then also an increase of the WNT-signalling molecule DVL2 and beta-catenin in patient samples. The authors used the B-ALL SUP-B15 cell line to further investigation of the interplay between CFTR and Wnt pathway. They demonstrate an interaction between CFTR and DVL2 by co-immunoprecipitation and show that knockdown of CFTR reduces the expression of DVL and MYC as well as impairs the proliferation of the cells. Using Xenocraft experiments with the SUP-B15 cells they further showed that inhbition of CFTR lead to decreased B-ALL and improved survival of the mice, supporting the potential of an CFTR inhibitor.

Overall the work is solid, and a worthwhile continuation of previous work. On the other hand it is not particularly novel, given that the interplay between CFTR and B-ALL as well as with WNT signalling has been described before. Also no mechanistic explanation of CFTR interferes with the WNT pathway is provided, besides that CFTR interacts with DVL2. The cell experiments were only performed with one cell lines, which limits the generality of the conclusion, given that the results may not hold true in other B-ALL cell lines. The experiments with the CFTR-inh172 has similarly aready been performed before by the same authors with T-ALL cell lines (PMID: 31541940). Thus, the authors mainly adapted their previous work to B-ALL cells, without providing much novel insights.

Minor comment
1) Personally, I think it is worth the show the Western from Supp Figure 1 in Figure 1A.

Reply 1): We have added Supp Figure 1 and related legend in Figure 1A in revision.

2) The first sentence in the results part (lane 178) should get improved.

Reply 2): We have fixed this sentence, hope to meet requirement (please see Page 9, line 184-198).

3) The second headline in the results (Lane 195) is weird. How can something be evolutionary conserved in patients? Perhaps better "The CFTR-Wnt pathway plays a role in ..."

Reply 3): The CFTR-Wnt pathway was uncovered by us using zebrafish model in previous work. We have fixed this sentence, hope to meet requirement (please see Page 9, line 201).

Reviewer B

The manuscript investigates the association of CFTR expression and childhood B-ALL and its potential as a therapeutical target. The authors showed increased CFTR levels in a small cohort of B-ALL patients samples compared to normal controls and a possible reduction of active B-catenin in knockdown CFTR B-ALL cell lines. The authors also performed in vivo experiments using NOD/SCID mice injected with SUP-B15 cells and treated with CFTR inhibitor. These mice showed better prognostic compared to control mice. The manuscript is clear and fits the journal scope. It should be of interest to readers seeking B-ALL therapeutic targets. However, it is necessary to analyze a bigger cohort in the future. I have a few suggestions to improve the manuscript.

Page 09, lines 178-183: This paragraph is not very clear. I suggest the authors rewrite these sentences so that the meaning is clear.

Reply: We have fixed this sentence, hope to meet requirement (please see Page 9, line 184-198).

Figure 1A:
- The authors say: “we collected the peripheral blood of human childhood patients diagnosed with primary B-ALL as well as non-leukemia controls (see Supplementary information, Tables S1 and S2 for control and patient information) and isolated lymphocytes” (lines 180-183) and refer to figure 1A at the end of the paragraph. However, figure 1 legend mentions bone marrows samples. Were the samples obtained from peripheral blood or bone marrow?
- How did the authors quantify the relative CFTR expression?

Reply: Thank you for pointing our mistake, the detected sample in Figure 1A was peripheral blood. We have fixed related text in revision (please see Page 9, line 184-194). We measured signal strength grayscale of western blotting assay using software ImageJ. All detected sample expression was relative to internal reference protein (please see Page 6, line 114-119).

Supplemental Figure 1: It is not clear if the samples are from peripheral blood or bone marrow.

Reply: Sorry for our unclear content. It is peripheral blood samples detected by Western blot related to Figure 1A. We have fixed related text in revision (please see Page 6, line 114-119; and Page 16, line 342-345). Reviewer 1 think it is worth the show the Western from Supp Figure 1 in Figure 1A, so we adjust the figure in revision.

Figure 2: I suggest the TOP/FOP luciferase reporter assay in siRNA CFTR SUP-B15 cells to show the reduced activation of WNT/B-catenin signaling.

Reply: We have added TOP/FOP luciferase reporter assay, please see revised Figure 2F. In addition, we have rearranged Figure 2 to improve the quality and optimize the resolution.

Supplemental Figure 2: In my opinion, it is hard to affirm that active B-catenin is reduced because the W.B signal is weak, even for the control sample. I believe the best way to show decreased B-catenin activation is by performing a luciferase assay.

Reply: We have also added TOP/FOP luciferase reporter assay, please see revised Supplemental Figure 1B. Reviewer 1 think it is worth the show the Western from Supp Figure 1 in Figure 1A, so we adjust the figure in revision.

Figure 3: The authors should indicate in the legend what the arrowhead means.

Reply: The arrowheads show the leukemia cells, we have fixed this issue in revision (please see Page 17, line 374).