Cover letter

Dear editor,

We are delighted to resubmit our revised manuscript entitled “VX-509 Attenuates the Stemness Characteristics of Colorectal Cancer Stem-like Cells by Regulating the Epithelial-Mesenchymal Transition through Nodal/Smad2/3 signaling” (Manuscript NO.: 89393, Basic Study) to *World Journal of Stem Cells*. We are very appreciative of the comments on the manuscript. Following these suggestions, we have modified our text accordingly. To facilitate the review of this revised manuscript, please note that we have highlighted the additions/changes with yellow color (revisions parts according to peer-review’s comment) and green color (language polishing parts and writing mistake correction parts) in the manuscript. Besides, we put the decomposable Figures in PowerPoint (We made the figures with Adobe Illustrator. If any problems in Figures, please contact us anytime.)

We appreciate in advance your timely attention to our revision.

With kind regards,

Yours Sincerely

Qiongying Hu

Response to reviewers' comments

The comments of reviewer's are valuable and comprehensive. We have revised the manuscript according to the comments point by point as follow:

This study provides interesting observation on a matter of significant biological and clinical relevance. However, the manuscript presents relevant problems in its present form.

1. In the Introduction section: (Lines 98-99 onwards): "As the traditional RA treatment, is the inhibitory effect of VX-509 on CRC stemness related to JAK3 or other pathways?" This sentence is totally misleading, and unplugged from the rest of the introduction section. In this regard, there is no introduction of the interplay and relevance between TGF-Beta/Nodal, Smad, and JAK3 within the context of colorectal tumorigenesis. The Authors should specify that other studies pointed at the issue of the relevance of JAK3 in colorectal cancer and CSCs, including the fact that other JAK3 inhibitors have been already shown to be effective in colorectal cancer treatment in in vivo animal model and in inducing apoptosis and colorectal cancer putative CSC cell cycle arrest in vitro. Among these studies: Novel……

Response: We have added new contents in the introduction (Line 98-101).

2. In the introduction section, a major part is lacking, that is explaining how the present study poses itself within the context of the already existing, above mentioned, literature, ad what are the main features of originality in the present study itself.

Response: We have added new contents in the introduction (Line 101-106).

3. (lines 60-61): "It is predicted that 3.2 million new CRC cases and 1.6 million deaths worldwide by 2040". Please modify: …that 3.2 million new CRC cases and 1.6 million deaths will occur worldwide…

Response: We have modified it (Line 62-63).

4. (line 63): "that is a long time for CRC patients to face". Change into: …"there is

still a long time….

Response: We have modified it (Line 64).

5. "which endows CSCs with the potential of cell proliferation and differentiation. Please change into: which endows CSCs with the potential of aberrant cell proliferation and differentiation…

Response: We have modified it (Line 71).

6. (Lines 74-75): "CSCs……provides". Change into: CSCs….. provide

Response: We have modified it (Line 76).

7. (lines 89-90): "A recent study demonstrated that Nodal specifically expressed in…". Change into: ……Nodal is specifically expressed…

Response: We have modified it (Line 90).

8. (Line 96): "A phase II clinical trial of VX-509 for the treatment demonstrated that…". Change into: A phase II clinical trial of VX-509 for the treatment of rheumatoid arthritis (RA demonstrated that….

Response: We have modified it (Line 96-97).

9. (line 141): "Washing for three times,…" Change into: after washing for three times…

Response: We have modified it (Line 146-147).

10. (Line 145): "106 cells suspended….". Change into:…cells were suspended…

Response: We have modified it (Line 155).

11. In the Methods Section, the Authors should specify the rationale for Aldefuor assay. ALDEFLUOR™ is a non-immunological fluorescent reagent system that has supported over 1000 publications for the detection of aldehyde dehydrogenase-bright (ALDHbr) cells in over 80 different tissues. High expression of ALDH has been reported for normal and cancer stem and progenitor cells of various lineages. The

Authors must specify why such an assay would be affordable enough to identify CSCs, when the kit itself reports that ALDH is also expressed in normal cells.

Response: We have added new contents in the methods section (Line 150-154).

12. (Line 214): "For vivo animal treatment…" Change into: …For in vivo….

Response: We have modified it (Line 223).

13. (line 259): "cDNA was reversibly transcribed…" Change into:…reverse-transcribed…

Response: We have modified it (Line 268).

14. (line 268): "Statistic"…Change into: Statistics

Response: We have modified it (Line 277).

15. (line 274): "Result"…Change into: Results

Response: We have modified it (Line 283).

16. (lines 330-331…): "EMT transformation drives cancer cells to dedifferentiate into CSCs cells by improving cell plasticity [22], we proposed that whether VX-509 exerts its CSCs stemness inhibitory effect by involving EMT process?". Please, rephrase as: Since EMT transformation drives cancer cells to dedifferentiate into CSCs by improving cell plasticity [22], we decided to investigate whether the inhibitory effect elicited by VX-509 on CSC may have involved changes in EMT patterning.

Response: We have modified it (Line 338-342).

17. (lines 333-335…): "Consistent with our hypothesis, VX-509 treatment repressed the expression of EMT related proteins (E-cadherin, N-cadherin and Vimentin) compared with mock group (Figure3H-I)". Please, integrate as it follows: A well-known hallmark of EMT is the upregulation of N-cadherin followed by the downregulation of E-cadherin. Consistent with our hypothesis, VX-509 treatment remarkably changed the expression of EMT related proteins, by eliciting an up-regulation of E-cadherin, while inhibiting the expression of N-cadherin, as well as

vimentin expression, as compared with the mock group (Figure3H-I).

Response: We have modified it (Line 343-346).

18. (lines 372-374), the Authors report "After treatment with VX- 509, gene expression levels were determined using qRT-PCR and the results showed that Nodal were increased in in HCT116 CSCs and HT29 CSCs compared to levels in their control cells (Figure 5C)". There are NO Data showing such an increase. Panel 5C shows a decrease in the gene expression of Nodal following cell exposure to VX- 509.

Response: We apologize for the mistakes. We have modified it (Line 384).

19. In Figure 5, panel E reports the densitometric analyses of Nodal and pSmad2/3 in HCT116 and HT29 CSCs treated in the absence or presence of different concentrations of VX-509. However, the legend of the same figure (line 779) reports that panel (E) is "The densitometry analysis of Oct4, Nanog, SOX2, normalized against GAPDH". The Western blot and the densitometric analyses of these proteins are not shown in the figure! These data must be presented in additional panels.

Response: We apologize for the mistakes. We have modified it (Line 817-818).

20. (lines 420-421): In the Discussion Section. "Somatic cells can pluripotent stem cells through transient ectopic overexpression of transcription factors such as…". A verb is missing: can transform into?

Response: We have modified it (Line 432).

21. (lines 524-526): "VX-509 treatment inhibited EMT-related phenotypic proteins, suggesting that its ability to reverse the activation of induced by Nodal stimulation and inhibit dedifferentiation processes in CCSCs". Please rephrase.

Response: We have modified it (Line 534-536).

22. Please, also rephrase the last part of the Conclusion section: "Moreover, VX-509 regulated the transcription and protein expression of Nodal and inhibited the phosphorylation of downstream protein Smad2/3 to prevent the EMT process of

CCSCs and inhibit continuous self-renewal of CCSCs and consequently reduced the generation of CCSCs, offering a potential clinical use of CRC". Change in: …. and consequently reduce the generation of CCSCs, offering potential clinical perspectives….

Response: We have modified it (Line 548-549).