Author Point-to-Point Reply Letter to Reviewers

Manuscript Title: NFkappa B Promotes the Stem-like Properties of Leukemia Cells by Activation of LIN28B

Manuscript Number: WJSC 38632

Reviewer reports:

Number ID: 03478635 Classification: Grade C (Good)

Language Evaluation: Grade B: minor language polishing

Conclusion: Minor revision

The manuscript describes about the stem-cell like properties of leukemia cells by activation of LIN28B. The Trypan Blue Exclusion method for Colony formation assay and serial replating assay should be described more in detail to indicate the cell numbers. The increase in LIN28B mRNA by transfection of pEGFP-LIN28B shown in figure 4B should be explained before the description of the results showing the colony number increase in LIN28 overexpressed cells compared to MG-132-treated cells. Colony number change in LIN28 overexpressed cells may be shown.

A: We thank the Reviewer for identifying some areas to be improved. We agree with the reviewer that Trypan Blue Exclusion method for Colony formation assay and serial replating assay should be described in more details. Now, we provide detailed description of these assays and cell numbers are indicated (Page 7, Line 3 – 6, Line 12 - 14).

As the reviewer suggested, we changed the order of Figure 4 and amended the text too. The legend of Figure 4 was revised accordingly.

The effect of LIN28B overexpression has been reported in a separated study and overexpression of LIN28B does increase the colony numbers. (Zhou J, et al. Mol Cancer Res. 2017 Mar;15(3):294-303).

Number ID: 00609371 Classification: Grade D (Fair)

Language Evaluation: Grade B: minor language polishing

Conclusion: Major revision

The major concerns are: 1)both bortezomib and MG-132 are fairly broad spectrum proteasome inhibitors. for example, MG-132 can activate c-Jun N-terminal kinase (JNK1), which initiates apoptosis. therefore, these inhibitors are not specific enough to support the conclusion. 2)Why overexpression of NF-κB in HEK393T (Human embryonic kidney cells), instead of in LSC, which might employee completely different regulatory mechanism?

A: 1) We thank the Reviewer for these two critical suggestion and conducted additional experiments by using a more specific NF-κB inhibitor, IKK-2 Inhibitor IV. The IKK-2 Inhibitor IV is a potent inhibitor of IKKβ (IKK-2) which interferes with NF-κB DNA binding (Karin, M., et al. 2004. *Nat. Rev. Drug Discov.* **3,** 17). So, the IKK-2 Inhibitor IV is a direct and specific NF-κB inhibitor. TF-1a cells were treated with a serial doses of IKK2 inhibitor IV. We observed increasing inhibition of cell proliferation starting from 0.625 μM to 10μM, with 50% inhibition at around 5μM. In agreement with the data derived from Bortezomib and MG-132, IKK-2 inhibitor IV treatment significantly reduced the mRNA and protein levels of LIN28B. In conclusion, the regulation of LIN28B by NFκB activity is specific. We added these contents into main text (Page 8, Line 12 – 17) and made a new Supplementary Figure S1.

2) HEK293 cells are easy to be transfected and have high transfection efficacy. So, they are very commonly used for transection experiments. Although LSC cells are ideal here, they are difficult to be transfected and grow in culture system. We have different experiments in different system to support our conclusions. Overexpression in 293T cells is only one part of experiments.

Number ID: 02446101 Classification: Grade B (Very good)

Language Evaluation: Grade B: minor language polishing

Conclusion: Accept

The authors investigated the relationship of NF-κB activity and LIN28B expression and their roles in leukemia stem cell (LSC)-like properties. The results show a regulatory signaling, NF-κB/LIN28B, which is important for the maintenance of leukemia stem cell-like properties and it might represent an attractive therapeutic target for effective treatment of AML disease. This is a interesting and useful paper. It really provide some important information to the readers. So, acceptance should be recommended.

A: We appreciate Reviewer's enthusiasm on our manuscript.