Reviewer's report

Title: Selective repression of RET proto-oncogene in Medullary Thyroid Carcinoma by a natural alkaloid berberine

Version: 2
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Reviewer: Lei Ye

Reviewer's report:

In this manuscript, the authors reported that berberine can stabilize the G-quadruplex structure formed on the RET promoter region, and suppress the transcription of RET in MTC. They also demonstrated that the repression of RET transcription by berberine resulted in the down-regulation of the PI3k/Akt pathway, which further inhibited the MTC cell proliferation through cell cycle arrest and the activation of apoptosis. If this is true, berberine, as a natural alkaloid, would be a potential drug for MTC therapy. However, I have two major concerns as followed.

Major Compulsory Revisions:
1. An important data in this manuscript is that the G-quadruplex structure mediate the inhibitory effect of berberine on RET, as berberine suppressed the expression of RET in MTC TT cells but not in PTC K1 cells, in which RET is under the control of the promoter region of CCD6 due to chromosomal rearrangement. Unfortunately, in fact, K1 cells harbored BRAFV600E but not RET/PTC mutation (Schweppe et al. 2008, JCEM), which means that the promoter of RET in K1 are WT with G-quadruplex structure. So if the experiment data in this paper was true, it implied that the suppression of RET transcription by berberine was not through G-quadruplex, but some other regulator which is specific in TT cells. Alternatively, K1 cell lines the author used may be contaminated with other cell lines.

2. In the results section, the first paragraph of “Effect of berberine on RET promoter activity” (Figure 4A), the luciferase in both HEK293-WT and MT1 decreased at the same level in the presence of berberine. As the G-quadruplex structure cannot formed in HEK293-MT1, these result also suggested that the repression effect of berberine was not through G-quadruplex structure. So the conclusions of this manuscript ( “G-quadruplex forming region and the stabilization of this structure play a critical role in mediating the repressive effect of berberine on RET transcription” ) are not suitable.

Minor Essential Revisions:
1. In the first paragraph of the results section, it mentioned that the Tm was increased to ~90°C under berberine treatment, while it was 85°C in Figure 2B. Data should be consistent in the manuscript.

2. As showed in figure 2A, the molar ellipticity seems decreased about 18% under berberine treatment (e.g. it was 1100000 in “RET control” but 900000 in
“RET berberine” at 20°C). This implied that berberine might cause changes on folding pattern of the G-quadruplex structure. To make clear this question, the CD titration spectra of RETWT at different concentrations of berberine could be did, just as the authors did in their previous work for another G-quadruplex interaction molecular (Shin YJ et al. 2014, Oncogene).

3. The authors found that suppression the expression of RET by berberine in TT cells results in the down-regulation of the PI3k/Akt pathway but not the MEK/ERK pathway. As TT cells harbored RETC634W mutation, I suggested examination the pathway alternations also in another MTC cell lines MZ-CRC1(with RETM918T ) to test whether the pathway specific was caused by the property of TT cells or general in MTC cells.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.