ANSWERING REVIEWERS

PEER-REVIEW REPORT 1

The reviewer did not ask any question.

PEER-REVIEW REPORT 2

1. The name of the title is different between the manuscript and attached letter for submission. The reviewer recommends to use "Hsa-microRNA-202-3p up-regulated in type 1 gastric neuroendocrine neoplasms and DUSP1 may be its target gene" as a title.

ANSWER:

The character count in submission system was limited so I changed this title to a short one when I submitted the manuscript. But now, for the reviewer recommends to use this title, it is our pleasure to change it back to "Hsa-microRNA-202-3p up-regulated in type 1 gastric neuroendocrine neoplasms and DUSP1 may be its target gene" as the title.

2. The author must analyze expression level of miR202-3p both in the tumor and non-tumor sections. Then, the author should create another figure to show the quantitative difference in expression.

ANSWER:

As a general rule, the result of qRT-PCR is described by fold change between the two groups, not the data for each group. In our opinion, it is not necessary to show the values for both groups because the experimental focus is on the trend of miR-202-3p content between the two groups (the tumor and non-tumor sections).

3. Discussion is too long.

ANSWER:

We accept the opinion that "the discussion is too long." We are willing to make adjustments as editorial office requirement. If possible, please tell us the

word limit in the discussion section, and we will shorten it as soon as possible and return it.

4. It would be better to delete second paragraph in Future work, because no data can prove relations between miR-202 and herbal medicine.
ANSWER:
Indeed, there is no data can prove relations between miR-202 and herbal medicine. This is exactly why we are motivated to study this subject in our future work. So we think it is appropriate to put this paragraph in Future work. Of course, there's no problem with asking us to delete it.

5. Legends for Fig. 4 and Fig.6 have no explanation. Please describe more details in every legend.
ANSWER:
Fig. 4
This figure shows the intersection of the predicted genes of miR-202-3p. TargetScan, microRNAorg and PITA are three popular and authoritative on-line available target gene prediction databases. 427, 4038 and 666 potential target genes of miR-202-3p were predicted by them respectively. There were 215 genes at the intersection of them.
Fig. 6
This figure shows the possible role of miR-202-3p/DUSP1 in the pathogenesis of type 1 g-NETs. Hypergastrinaemia leads to ECL cell proliferation. We speculate that when DUSP1 is downregulated by high miR-202 expression, ECL cells will have more opportunities to develop type 1 g-NETs.

PEER-REVIEW REPORT 3

1. The abbreviation of NEN needs to be revised to NET. Additionally, NETs type 1 are not really considered as really malignant tumor and makes rare metastasis.
ANSWER:

As far as we know, NEN is short for neuroendocrine neoplasm. It includes NET (neuroendocrine tumors) and NEC (neuroendocrine carcinoma) two concepts. When referring to gastric neuroendocrine neoplasms in general terms, it must be represented by NEN, not NET, because it includes types 1, 2 and 3 as well as type 4 (NEC). When it comes to type 1 gastric neuroendocrine tumor, it's all right to use NEN or NET.

Although type 1 gastric neuroendocrine tumor makes not so much metastasis, it is definitely a kind of malignant tumor.

2. Abstract needs to be more descriptive to the key data/numbers etc.

ANSWER:

We accept this suggestion and would like to revise the "results" part of the abstract to read as follows:

Six miRNAs (hsa-miR-194-3p, hsa-miR-202-3p, hsa-miR-6752-3p, hsa-miR-6800-3p, hsa-miR-6889-3p, hsa-miR-933) were significantly upregulated or downregulated in the tumours compared to the control samples. Among them, miR-202-3p was extraordinarily upregulated. RT-PCR of seven sample sets confirmed that miR-202-3p was upregulated in tumour tissues. In total, 215 target genes were predicted to be associated with miR-202-3p. Among them, dual-specificity phosphatase-1(DUSP1) was reported to be closely related to tumour occurrence and development. The dual-luciferase reporter assay showed the relative activity of the wild-type group was decreased by 52% compared with that of the control group, which means miR-202-3p directly regulated DUSP1.

3. Limitation in design: inappropriate control samples with high risk of bias, definition of NET is not provided, limited number of samples 3 for array and 7 for validation.

ANSWER:

We acknowledge that our sample size is small, but in fact, due to the low incidence of neuroendocrine tumors (the annual incidence is about 6.98 / 100

000, whereas type 1 gNETs account for less than 5% of them), it is almost impossible to get as many samples as common oncology studies. MiRNA-202-3p in 3 groups of tumor were significantly high expression. This phenomenon can provide direction for the next steps of research (qRT-PCR and target gene prediction).

Definition of NET is provided in the beginning of the article: Gastric neuroendocrine neoplasms (g-NENs) are a rare malignancy mainly derived from enterochromaffin-like (ECL) cells and occasionallyderived from other cells that secrete somatostatin, auxin or serotonin.

4. The functional data to miR-202-3p are to preliminary and simple luciferase assay seems to my point of view insufficient to show the function role.

ANSWER:

The results of a luciferase reporter assay show that the relative activity of the wild-type group was decreased by 52% compared with that of the control group. And, compared with the wild-type group, the relative activity of the mutant group was increased 44%. This phenomenon can make it clear that DUSP1 is directly regulated by miR-202-3p.

There are indeed some other experimental methods to further study the interaction between microRNAs and target genes, which will be our further research goal.

5. Conclusions are not supported by the data.

The conclusion of the article is as follows: miR-202-3p is upregulated in type 1 g-NEN lesions and might play important roles in the pathogenesis of type 1 g-NENs by targeting DUSP1.

ANSWER:

We have confirmed by data that miR-202-3p is upregulated in the lesions. The speculation on the role of the two is based on the prediction of bioinformatics and the experimental validation of tool cells. Therefore, we think our data can support the above conclusion.