Dear Reviewer,

Thank you very much for your encouraging letter together with the constructive critiques regarding our manuscript "MiR-422a is an independent prognostic factor and functions as a potential tumor suppressor in colorectal cancer" (ESPS Manuscript NO: 25505). As you suggested, we have revised the manuscript according to your recommendations. Track changes are used in the revised manuscript. Our responses to the comments are as follows:

Comments:

1. Were tissues from patients used as a whole or were any attempts done to dissect only carcinoma cells/ areas? For the normal controls were any attempts done to take only mucosa for the experiments or whole tissue was used which would include other tissues? Authors should comment.

Response to Reviewer comment: We have used cancer tissues as a whole which were carefully confirmed by pathologists. And we tried to take only mucosa tissues for the controls in this experiment, because CRC is a disease originating from glandular epithelium of the colorectal mucosa. We have illustrated the related content in "MATERIALS AND METHODS" of the revised manuscript.

2. Were any verification done that the mimetic transfection was successful? If yes it should be presented or mentioned, if no, it should be done. The levels of miR422 endogenously expressed in the two cell lines should also be investigated. Moreover such expression could be knocked down by RNAi to check if effects opposite to those seen with exogenous hyper-expression would be observed. These would greatly enhance the credibility of currently presented results.

Response to Reviewer comment: Thanks for your valuable recommendation. In our preliminary experiments, we have observed that the levels of miR-422a endogenously expressed in several CRC cell lines, including HT-29, SW480, SW620 and HCT-116. The results showed that the levels of miR-422a were quite low in these CRC cells, and there were no significant differences in the expression level of miR-422a among these cells (data not shown). Thus, we transiently up-regulated the expression of

miR-422a in two CRC cell lines by transfection with miR-422a mimics without knocked down by miR-422a RNAi. The effect of transfection was evaluated by transfection efficiency assays. We have added corresponding experimental data to the revised manuscript. The corresponding contents were shown in "Result (Over-expression of miR-422a inhibited CRC cell proliferation)" section of the revised manuscript.

3. In figure 2 the p value is different from the p value for mir422 in the univariate analysis of table 2. Shouldn't they be the same? Please check.

Response to Reviewer comment: Thanks for your reminder. The p value in figure 2 and table 2 were calculated by Kaplan-Meier survival analysis and Cox regression univariate analysis, respectively. Both the parameter estimation method and hypothesis testing method are different in the two different analyses. Therefore, we obtained different p values.

4. In table 1 units of expression should be provided.

Response to Reviewer comment: The expression of miR-422a was measured using relative quantitative rather than absolute quantitative PCR. Therefore, there is no unit for miR-422a expression.

5. In table 2 the variables should be defined. For example what is the comparison in the TNM group? I and II versus III or I versus II and III etc.

Response to Reviewer comment: Thanks for your suggestion. We have complemented variables categories in the revised Table 2.

6. It appears from fig 4 and 5 that the cultures may have some type of yeast contamination. Authors should check and repeat the experiments in sterile conditions to confirm validity.

Response to Reviewer comment: Thanks for your recommendation. We have consulted a microbial expert named Wei Li who worked in Shandong University about the yeast contamination in fig 4 and 5. He concluded that there seemed no yeast contamination in the two pictures. The oval things which were dyed and looked like yeast were those cells that could not completely through and were blocked in the holes.

7. In fig. 3, 4 and 5 the number of experiments performed to derive the confidence intervals presented should be mentioned.

Response to Reviewer comment: Thanks for your recommendation. All assays were performed in triplicates to derive the confidence intervals. We have added the content in figure legends.

8. In addition to the presented analysis in fig 2 based on the median, another analysis of survival could be of interest, based on the change of expression compared to adjacent normal mucosa (decreased, no change (-1 to +1), increased).

Response to Reviewer comment: Thanks for your recommendation. We have complemented analysis of survival based on the change of miR-422a expression compared to adjacent normal mucosa. The result was shown in result section and Fig 2B in the revised manuscript.

9. Some editing is needed. I suggest, for example, that “para-carcinoma” be replaced by normal adjacent to carcinoma”.

Response to Reviewer comment: Thanks for your recommendation. We have replaced para-carcinoma by normal mucosa adjacent to carcinoma in the revised manuscript.

In summary, we thank editors and the reviewers for your insightful and constructive analyses of this work. In this revised manuscript, we have addressed all your concerns thoroughly.

Sincerely yours,

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