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Low expression of CDK5RAP3 and DDRGK1 indicates a poor prognosis in Patients with Gastric Cancer

Dear editors and reviewers:

We are grateful to you for your valuable comments and suggestions, which help us to improve the quality of the manuscript. We have study the comments carefully and have made modifications and corrections which we hope meet your approval. We have revised the manuscript according to your helpful advices and detailed suggestions. Below are the descriptions of our revision.

Comments to the Author:

Reviewer #00068723:

The authors investigated CDK5RAP3 and DDRGK1 in gastric cancer tissues with clinicopathological data. They found that expression patterns of the two proteins were related with differentiation of cancer cells, depth of invasion, lymph node metastasis, and prognosis. The results were potentially useful. But the definition was not clear of high or low expression of the two proteins with immunohistochemistry. The definition may affect the results. Figure 1 D. High expression panels showed positive signals in fibrous or stromal tissues, outside the cancer cells. Low expression panels showed weak positive signals in fibrous tissues. This difference of staining patterns was a crucial point of this study. It would be better to define high or low expression in words. It would be appropriate to show results in scores of staining patterns. For example, score 1, 2, 3, and 4 following the intensity of positive signals.

Response: Thank you for your comments and suggestions on this study. The definition for the evaluation of CDK5RAP3 and DDRGK1 staining intensity was as follows: no staining (score of 0), weak staining (light yellow, score of 1),

moderate staining (yellow brown, score of 2) and strong staining (brown, score of 3). The positive proportion of stained tumor cells was scored as follows: \leq 5% positive cells (score of 0), 6% to 25% positive cells (score of 1), 26% to 50% positive cells (score of 2), \geq 51% positive cells (score of 3). If the total scores (intensity multiply percentage score) was less than 3, the protein expression was considered low, however, if the score was 4 or higher, the protein expression was considered high. We have added this information in the Method section with red color.

According to the reviewer's suggestion, we have changed the Figure 1D to show results in scores of staining patterns (score 0, 1, 2, and 3 following the intensity of positive signals).

Reviewer #02558978:

Manuscript ID: 39852 Manuscript title: Low expression of CDK5RAP3 and DDRGK1 indicates a poor prognosis in Patients with Gastric Cancer

The manuscript is acceptable

Minor comment: 1. Page 6, the sentence, "It was found that the expression of CDK5RAP3 and DDRGK1 in 6 patients was higher than that in cancer tissues (Fig. 1A)." did not specify which region with higher expression of CDK5RAP3 and DDRGK1 in 6 patients and is needed to modify.

Response: We have modified this sentence in the manuscripts with red color as following: It was found that the expression of CDK5RAP3 and DDRGK1 in 6 patients was higher in respective adjacent non-tumor tissues than that in gastric cancer tissues (Fig. 1B).

2. The results indicated that the expression of CDK5RAP3 and DDRGK1 in gastric cancer cell lines decreased with the decrease of differentiation degree of the gastric cancer cell lines. Authors may further manipulate both gene expression in different gastric cancer cell lines, such as overexpression or knockdown, and investigate the characteristics of cancer cells to provide more

molecular evidence by in vitro cell model. Then, these data will further support the conclusion that low expression of CDK5RAP3 and DDRGK1 act as the prognostic factors for gastric cancer patients.

Response: Thank you for the reviewer's helpful advice. In this study, we focus on the effect of expression patterns of CDK5RAP3 and DDRGK1 on the clinicopathological characteristics and the prognosis of patients with gastric cancer. We found that expression patterns of the two proteins were related with depth of invasion, lymph node metastasis, and prognosis. These results were potentially useful. As the reviewer's kind suggestion, this study can be considered as a previous basis research; we will further manipulate both genes expression in different gastric cancer cell lines, and investigate the characteristics and mechanism of these genes effect on gastric cancer. We have added this information in the "Discussion" section with red color.

Reviewer #02977366:

In the manuscript entitled "Low expression of CDK5RAP3 and DDRGK1 indicates a poor prognosis in Patients with Gastric Cancer", the author found that co-expression of CDK5RAP3 and DDRGK1 can provide a more accurate model for the long-term prognosis of gastric cancer. In my opinion, the study discovery the potential value of CDK5RAP3 and DDRGK1 in gastric cancer, this study is full of innovation and clinical translational value. Comment:

1. In the text, author mentioned that expression of DDRGK1 was correlated with the sex of the patients ($P=0.080$), the P value beyond the cutoff set by the author in the section of statistical analysis ($p < 0.05$).

Response: The P value of the sex of the patients ($P=0.080$) beyond the cutoff set ($P=0.05$). We have corrected it in the manuscripts with red color.

2. CDK5RAP3 and DDRGK1 are key genes which may participate in the biological regulation of neoplasms, while the detail mechanism of them in gastric cancer are still less clearly. Author showed that the co-expression of

CDK5RAP3 and DDRGK1 can provide a more accurate model for the long-term prognosis of gastric cancer. We suggested that the author should clarify the regulation relationship between CDK5RAP3 and DDRGK1 in GC cell lines, whether they interact with each other, which is the main regulator and whether the ufmylation of DDRGK1 could be influenced by the expression of CDK5RAP3.

Response: Thanks for the reviewer's suggestions. In our previously study, we found that CDK5RAP3 and DDRGK1 can bind to each other by co-immunoprecipitation combined with mass spectrometry (this part we already added in the Supplementary Figure 1). As a result, we investigated the effect of expression patterns of CDK5RAP3 and DDRGK1 on the clinicopathological characteristics and the prognosis of patients with gastric cancer in this study. As the reviewer suggested, after this previous basis research, we will further investigate the mechanism of interaction between CDK5RAP3 and DDRGK1 and its effect on gastric cancer. We have added these in the "Results" and "Discussion" section with red color.

3. Author only compared the prognostic value of high level of CDK5RAP3 and/or DDRGK1 with low level of CDK5RAP3 and DDRGK1. We suggest to compare the prognostic value of high CDK5RAP3/ low DDRGK1 with low CDK5RAP3/ high DDRGK1, in order to identify the relationship between them.

Response: We have compared the prognostic value of high CDK5RAP3/ low DDRGK1 to low CDK5RAP3/ high DDRGK1, and there were no significant difference between these survival curves (Supplementary Figure 2). We have added this in the "Result" section with red color.

In conclusion, we have checked the manuscript and revised it according to all the comments. We submit here the revised manuscript as well as a list of changes. If you have any question about this manuscript, please don't hesitate

to let me know.

Sincerely yours,

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