Author's response to reviews

Title: Meta-analysis shows that circulating tumor cells including circulating microRNAs are useful to predict the survival of patients with gastric cancer

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Author's response to reviews: see over
Dear reviewers,

Thank you very much for your comments. We have revised the manuscript after serious considering of them. Revised portions and any relating corrections have been marked in red in the updated manuscript. We hope the comments could be satisfied. Thank you again for your constant interest in our work.

Below are our specific responses to the comments.

**Comments from Reviewer Zhen-Ning Wang**

1. In my opinion, the authors did not well solve the problem on redundant studies based on potential same patients’ population, such as in inclusion criteria: “(3) studies from the same institutions were included if they reported different markers or applied different methods”. I am afraid that there is still considerable impact caused by redundant studies. The authors should try to conduct adequate and in-depth analyses to eliminate the impact of redundant studies.

**Response 1:**

We agree with the reviewer. To further assess and get rid of potential biases by reports of same institutions, we have added several analyses. First of all, we considered the studies by Arigami [32], Arigami [36], Arigami [42], and Uenosono [48] were reported from the common organization. The situation was similar to ref Wu[25] and Uen[26]. So, we made sensitivity analyses by including only the latest reports (ref [42] and ref [25]) from the same research institutions, yielding an overall effect which was
quite close to that without exclusion of any of the references mentioned above (RFS: HR=2.71, 95%CI [1.72-4.27], \(I^2=51.42\%\), n=9; OS: HR=1.88, 95%CI [1.48-2.40], \(I^2=42.07\%\), n=24). (line 126-129, line 230-233)

Secondly, we tested whether inclusion of these 6 studies had brought in any biases by subgroup analyses. Removing all of the 6 studies did not contribute to significant changes of the pooled measures (for OS: the same institution, HR=1.55, 95%CI[1.23-1.96], n=8, different institution, HR=1.93, 95%CI[1.53-2.44], n=22; for RFS, different institution, HR=2.71, 95%CI [1.72-4.27], n=9) although there was a tendency that the 6 studies from the same populations were likely to present homogeneous results (for OS, same vs. different, \(I^2=0.00\%\) & P=0.901 vs. \(I^2=46.20\%\) & P=0.010). (line 233-241)

Lastly, we measured the biases of inclusion of these studies by meta-regression. However, the results showed that it was not able to demonstrate any significant influences based on our available data (RFS: slope=1.4738, SE=0.9603, p=0.1249; OS: slope=-0.0064, SE=0.1350, p=0.9623). (data not shown in manuscript)

Therefore, the results of our additional analyses indicated that using of the other 4 studies (ref [26], ref [32,36,48]) of similar institutions did not lead to significant impact. Nevertheless, it did suggest that future studies could benefit from recruitment of homogeneous populations.

2. To further validate the reliability of results, the authors should conduct in-depth analysis with/without these studies on microRNA.
Response 2:

We agree with the reviewer. To make sure, we further made subgroup analyses and meta-regression according to marker type of miRNA or the other sources. In subgroup analyses, removal of studies with or without miRNAs did not bring out obvious changes in overall effects (OS: non-miRNA group, HR=1.80, 95%CI[1.45-2.25], n=22, miRNA group, HR=1.70, 95%CI[1.33-2.16], n=8; RFS, non-miRNA group, HR=3.08, 95%CI[1.80-5.26], n=9). But it indicated a need for standard markers to identify CTCs since studies with only miRNAs were more consistent than the other studies which contained many different kinds of markers (OS: miRNA group vs. none, I²=0.00% & P=0.602 vs. I²=41.33% & P=0.023) (line 129-133, line 204-207, table 2). Furthermore, in meta-regression, inclusion of studies with miRNAs did not cause significant heterogeneity (RFS: slope=-0.2650, SE=0.3991, P=0.507; OS: slope=0.1061, SE=0.1386, P=0.444), suggesting that it was also doable to combine the results of studies with and without miRNAs (line 129-133, line 221-223 and table 3).

Comments from reviewer :Nuh N Rahbari

1. The authors have addressed my comments appropriately. However, I do not agree with the reviewers that miRNAs should be considered to indicate CTC. They just represent biologically very different entities that need to be dealt with separately. While I would accept that studies on CTC and miRNA are pooled in separate forrest plots, I would not accept pooling these studies in the same plots. The authors should
either remove studies on miRNA completely or include them in separate meta-analyses. If the authors decide to keep the studies on miRNA in their manuscript they should clearly indicate this in the title and throughout the paper to make sure CTC and miRNA are not different biological entities.

Response 1:

Thank you! We quite agree with the reviewer that circulating miRNAs were mostly different from CTCs, biologically. However, we kept the studies with miRNAs in our meta-analyses in order to maintain the maximum information. We have treated the miRNAs as novel markers for CTCs in our manuscript and added compatible revisions.

Firstly, we revised the manuscript title to indicate that circulating miRNAs were also analyzed in our meta-analysis (please see the title of the manuscript).

Secondly, we indicated in the manuscript that circulating miRNAs were regarded as special markers for CTCs (please see line 129-130).

Thirdly, because miRNAs were different in nature and not as specific as the other markers, we further performed separate meta-analyses to make sure whether there were any differences in total effects of studies with and without miRNAs. In subgroup analyses, removal of studies with or without miRNAs did not bring out obvious changes in overall effects (OS: non-miRNA group, HR=1.80, 95%CI[1.45-2.25], n=22, miRNA group, HR=1.70, 95%CI[1.33-2.16], n=8; RFS, non-miRNA group, HR=3.08, 95%CI[1.80-5.26], n=9). But it indicated a need for standard markers to identify CTCs since studies with only miRNAs were more consistent than the other
studies which contained many different kinds of markers (OS: miRNA group vs. none, $I^2=0.00\% \ & P=0.602$ vs. $I^2=41.33\% \ & P=0.023$) (please see line 129-133, line 204-207 and table 2).

Finally, we also performed meta-regression to confirm any biases by inclusion of researches on miRNAs. In meta-regression, inclusion of studies with miRNAs did not cause significant heterogeneity (RFS: slope=-0.2650, SE=0.3991, $P=0.507$; OS: slope=0.1061, SE=0.1386, $P=0.444$), suggesting that it was also doable to combine the results of studies with and without miRNAs (please see line 129-133, line 221-223 and table 3).

In summary, all the analyses made us to conclude that CTCs as well as circulating miRNAs could predict the survival of patients with gastric cancer.