Author's response to reviews

Title: Macrophage inhibitory cytokine 1 (MIC-1/GDF15) as a novel diagnostic serum biomarker in pancreatic ductal adenocarcinoma

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Author's response to reviews: see over
Cover letter for BMC Cancer

Dear Prof. Oliver Stoeltzing and Ms. Roselyn Remoto,

Thank you so much for the e-mail and the reviewers’ comments on our manuscript (MS: 3540677671000148). In response to the reviewers’ comments, we revised our manuscript to address the reviewer’s concerns. Now, I resubmit the revised version of our manuscript to you for consideration for publication in BMC Cancer. The detailed responses to the reviewers’ comments are as follows:

Reviewer #1’s comments:

Several areas require clarification to assist the reader. In particular, there is missing data in the text. When possible, all the results in the text should be described as mean ± standard deviation.

As the reviewer suggested, we carefully checked the entire manuscript for missing data and corrected it in the revised manuscript, we also changed the expression style of the results to the “mean ± standard deviation” as the reviewer pointed out in the revised manuscript.

1) Quantification of MIC-1 mRNA by PCR was done from what type of tissue (Frozen or paraffin)? Please indicate in the methods.

Quantification of MIC-1 mRNA by PCR was done from frozen cancerous and matched normal tissues.

As the reviewer suggested, we added the following information in the methods:

“of cancerous and matched normal tissues”

2) Please describe the y axis in the legend in Figure 1a. What is being measured on the Y axis?

The numerical values of Y axis represents the MIC-1 mRNA expression level of tissues which determined by RT-PCR, and described as $2^{-\Delta Ct}$.

As the reviewer suggested, we added the following information in the revised legend of Figure 1a.

“(y-axis: the MIC-1 mRNA expression level, described as $2^{-\Delta Ct}$ with log10 scale axis)”

3) In the first paragraph of the results—where is the data for this sentence—“increased expression of MIC-1 was not significantly correlated with TNM”?

Since the difference was not statistically significant, so we do not describe the data in text and figure.

As the reviewer suggested, we list the data in the result section of revised manuscript.

4) For figure 1b—describe data as mean +/- standard deviation in the text.

As the reviewer suggested, we described the data of figure 1b as mean ± standard deviation in the revised manuscript.
5) What does the MIC-1 upregulated tumor refer to in Figure 1C—what is definition of MIC-1 upregulated tumor?
“Upregulated tumor” in this paper means that the expression of MIC-1 in tumor tissues is higher than that of adjacent tissues. As the reviewer pointed out, we carefully checked the misleading statements and added the following information to clarify the confusion in the revised manuscript. “with upregulated expression of MIC-1 in tumor tissues”

6) Figure 1d-- describe data as mean +/- standard deviation in the text.
As the reviewer suggested, we described the data of figure 1d as mean ± standard deviation in the revised manuscript.

7) For figure 1e-- describe data as mean +/- standard deviation in the text.
As the reviewer suggested, we described the data of figure 1e as mean ± standard deviation in the revised manuscript.

8) Add standard deviation to data in the text for serum MIC-1 in validation group.
As the reviewer suggested, we added the standard deviation to data of serum MIC-1 in validation group in the revised manuscript.

9) Data for figures 4a,b,c,d,e,f need to be presented as mean +/- standard deviation in the text.
As the reviewer suggested, we described the data of figure 4a,b,c,d,e,f as mean ± standard deviation in the revised manuscript.

Reviewer #2’s comments:

Questions:
1) What may be the underlying biology that could explain the better performance of MIC-1 in early-stage PDAC in comparison with CA19.9?
It is general thought that serum CA19.9 was significantly correlated with tumor stage of PDAC, especially tumor size. Therefore, the levels of serum CA19.9 may not significantly elevated in small early stage of PDAC, and the sensitivity is difficult to meet expectation accordingly.

In our paper, we found that elevated levels of serum MIC-1 are detectable in the earliest stages of PDCA, and tend to be raised independently of pathologic tumor stage and lymph node spread, suggesting that high levels of serum MIC-1 are not solely due to tumor burden, despite the expression and secretion of MIC-1 in the tumor tissue is Indeed significantly increased. Previous study have showed that MIC1 overexpression could results in 20-fold increase in serum MIC-1 levels in xenograft models bearing tumors secreting various engineered forms of MIC-1 (1), while the
description of MIC1 as a tumor marker in all reported literature did not point out a clear sources of serum MIC1 in tumor patients as yet. Elevated levels of serum MIC1 in early stage of tumor may be constituted of tumor cells overexpression, tumor micro-environment adaptive response and systemic immune stress response. This may be the underlying biology that the performance of MIC1 in early diagnosis of PDAC is superior to CA19.9.


2) If MIC-1 is influenced by inflammatory processes, as the authors mentioned, and is not able to distinguish between PDAC and chronic pancreatitis and moreover, high levels of expression are also observed in other neoplasias, how could be applied to screening of PDAC considering such inespecificity?

In our study, most patients with PDAC had elevated MIC-1 levels, even patients with small early stage cancers, suggesting that serum MIC-1 could be particularly helpful in the early detection of pancreatic cancer. And we found that serum MIC-1 is a more sensitive marker of PDAC than CA19-9. Although promising, MIC-1 has limitations as a serum marker for screening PDAC.

Firstly, Like CA19.9, serum MIC-1 levels are often elevated in the setting of pancreatitis. Indeed, CA19.9 was less commonly elevated in patients with pancreatitis in this study than was MIC-1. These results indicate that MIC-1 may not useful for distinguishing pancreatic cancer from chronic pancreatitis. Additional studies are needed to determine whether the screening utility of MIC-1 could be improved further by combining MIC-1 not only with CA19.9 but also with other serum markers that have shown some promise as pancreatitis markers. In addition, while MIC-1 is influenced by other inflammatory disease, serum MIC-1 levels are stable over time in patients with PDAC. It is possible that serial measurements, showing increasing serum MIC-1 levels over time, may be much more informative for early PDAC than a single measure.

Secondly, in addition to patients with PDAC, patients with several other cancers show elevations of MIC-1, suggesting that serum MIC-1 measurement may not suitable for single-species tumor screening. Even so, a simple noninvasive serum MIC-1 test could aid in the pancreatic surveillance of populations at high-risk, such as those with a strong family history of pancreatic cancer. And further, 65.1% of patients with early stage PDAC in our study had elevated MIC-1 and 78.1% had elevated MIC-1 or CA19.9, the measurement of these markers could potentially be used in conjunction with pancreatic imaging and other clinical information to help rule out a diagnosis of PDAC in high-risk. Besides the screening value in high-risk groups, given that MIC-1 levels were not highly elevated in patients with pancreatic benign tumor; MIC-1 levels may be useful in differentiating benign from malignant pancreatic tumor in specific circumstances. For example, in patients with comorbid disease, the distinction between a relatively benign neoplasm and an invasive cancer can spare
the patient the need for surgical exploration.

**Minor essential revisions**

Correct drafting errors at lines: 210: 723.9pg/mL it should be 723.9 pg/mL, by separate ; 211: 1192.6.pg/mL; 212: 1449.1pg/mL and so on.

As the reviewer suggested, we carefully checked the entire manuscript and corrected abovementioned mistakes in the revised manuscript.

**Line 137: It should be MIC-1, not MIC**

As the reviewer suggested, we corrected abovementioned mistake in the revised manuscript.

**Line 200: What does it mean “unregulated” in the context of this paper?**

**Down-regulated?**

“unregulated” in this paper means that the expression of MIC-1 in tumor tissues is not higher than that of adjacent tissues. As reviewer pointed out, “down-regulated” is more suitable in this paper.

As the reviewer suggested, we replaced “unregulated” with “down-regulated” to clarify the confusion in the revised manuscript.

**Line 273: ”Pancreatic Carcinomas” should not be capitalized. At line 275 it used correctly. Fix this and other terms along the paper.**

As the reviewer suggested, we carefully checked the entire manuscript and corrected abovementioned spelling mistakes in the revised manuscript.

Once again, thank you for your excellent and efficient editorial work, and the reviewers’ suggestive comments. We hope that our revised manuscript will meet the standard of publication in *BMC Cancer*.

Sincerely yours,

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