13 February, 2014

Manuscript: (6830) "Novel Therapeutic Targets for Pancreatic Cancer"

Dear Editor,

Thanks for the reviewing and comments on our manuscript. We take all the comments seriously and have addressed all the issues raised by the Reviewers. In the following context, we are presenting our response to Reviewers' comments point-by-point.

Comments from the Editors:

The review article was originally lack of provocative thoughts in the novel targets of pancreatic cancer, insufficient elaboration on the translational significance of the novel targets mentioned, omitting a conclusion of the overall therapeutic targets mentioned in the article, and requiring further elucidation for the reasons supporting the mentioned targets have therapeutic value in pancreatic cancer, (i.e. CTHRC1, HER3).

Responses: As advised by the Editors, we have elaborated on how the novel targets contributes to tumour activities of pancreatic cancer, and the anti – tumour activities of these targets brought up upon over/down – regulation which can be possibly used to treat PDAC patients. Besides, we have ended the article with a summary and a short summary at the end of each sections in the article, summarizing the importance of the targets mentioned in the article. The aim of introducing numerous examples is to led readers have an idea of the complex and diverse network of the signaling molecules in contributing tumour phenotypes expression, limitations of these targets under studies and to explore the plausibility to overcome them when combining with other targeted therapies.

Reviewer 00058434:

1. There are several spelling mistakes and grammatical expression error in the manuscript.

   Responses: We apologize for committing these careless mistakes and thank you very much for spotting them out, we have paid closer attention on these mistakes, and trying to break down lengthy sentences in reducing expression error.

2. It is necessary to define all the acronyms upon first time appearance in the manuscript.

   Responses: During revision we have stated the full name of the acronyms in the manuscript at their first time of appearance, and the official full name is followed with its acronyms in brackets.

3. More evidence have to be provided to demonstrate CTHRC1 to be a novel therapeutic valuable in pancreatic cancer.

Responses: CTHRC1 elevated expression would enhance the activation of Erk and Src signaling pathways which are known for facilitating tumour progression and tumour motility, and repress collagen I production which favors cancer metastasis. These suggested the CTHRC1 is a tumour enhancing gene and suppression of CTHRC1 has suppressed tumour metastasis and tumour motility in cellular model, therefore, we suggest that CTHRC1 is therapeutic valuable in preventing pancreatic cancer metastasis and deserve more studies of its efficacies in animal model by CTHRC1 suppression and combinatorial therapies with current chemotherapies in pancreatic cancer and other targeted therapies.

4. Further elaboration on how tyrosine kinase inhibitors (TIKs) can inhibit HER3, as HER3 do not have tyrosine kinase activity.

   Responses: In the revised version, we have mentioned HER3 activation requires another HER receptor which possesses tyrosine kinase activity in the HER family. Thus TIKs can inhibit activation of HER3 and preventing the activation of its downstream effectors which are oncogenic, e.g. PI3K/AKT signaling is reduced upon TIKs administration.

5. The manuscript lacks an overview of all the targets mentioned.

   Responses: In the revised version, we have ended the article with a summary summarizing the targets that have mentioned in the manuscript by exploring the plausibility in multiple targeted therapy, in which blocking pancreatic tumour metastasis, sensitizing tumour to chemo – therapies, enhancing drug penetration, etc. Besides, we have suggested to invest more in screening inhibitors using traditional Chinese Medicine (TCM) as the source, so as to obtain less toxic candidates.

   The content of the Summary in the revised manuscript is as follow:

   "A range of therapeutic targets in PDAC have been briefly described in this article, in which their anti – tumour and oncogenic activities are characterized through various experiments and can be taken as potential target for PDAC therapies development.

   Nevertheless, numerous of the targets are found overlapped with each other in producing certain kinds of tumour phenotypes, e.g. over – expression of CXCR4, Rac1, BMI1, and etc., in pancreatic tumour cell have observed a metastasis enhancement. In light of this, and hypothetically, in order to prevent metastasis, suppressing these targets should have a more pronounced effect in metastasis inhibition. Moreover, such outflanked approach may also prevent the tumour cell from switching into other signaling pathways producing the same tumour phenotypes, and achieving elimination ultimately. Besides, the current knowledge of each of these targets is insufficient, categorizing these targets by the tumour phenotypes produced and identify if there is any relationship between them and understand the mechanism behind, would allow the discovery of linkages among them in terms of proteins and mRNA expression levels and functional activations.

   Last but not least, screening of suitable inhibitors for these targets is crucial in putting these targets into practice. Toxicity of some of the inhibitors mentioned is reported, while,

traditional Chinese medicine (TCM) may be a good source for screening inhibitors that are less or non – toxic compounds, e.g. an EZH2 inhibitor, davidiin, is extracted from TCM *Polygonum capitatum* without toxicity observed in xenograft model [181].

Single effort from one side is far from enough in pancreatic tumour elimination due to its high malignancy and complex tumour microenvironment, multiple targets have to be considered in developing PDAC therapies, therefore, the way of applying these targets and which targets should be applied require further effort."

Reviewer 00071702:

1. A description of the manuscript outline should be provided in the introduction.

   Responses: Thank you very much for the advice, we have stated the content of the manuscript in the later part of the introduction as follow:
   "Therefore, in the following context of this review we are going to briefly evaluate plausible therapeutic targets, in terms of the molecular and cellular level which covers the roles of several signal transducers, signaling pathways, surface proteins, receptor proteins, non – coding RNA, epigenetic modifiers and tumour microenvironment in driving pancreatic cancer and to explore any possibilities of combinatorial therapy among them."

2. The manuscript does not have a concluding segment.

   Responses: In the revised version, we have ended the article with a summary summarizing the targets that have mentioned in the manuscript by exploring the plausibility in multiple targeted therapy, in which blocking pancreatic tumour metastasis, sensitizing tumour to chemo – therapies, enhancing drug penetration, etc. Besides, we have suggested to invest more in screening inhibitors using traditional Chinese Medicine (TCM) as the source, so as to obtain less toxic candidates.

   The content of the Summary in the revised manuscript is as follow:

   "A range of therapeutic targets in PDAC have been briefly described in this article, in which their anti – tumour and oncogenic activities are characterized through various experiments and can be taken as potential target for PDAC therapies development.

   Nevertheless, numerous of the targets are found overlapped with each other in producing certain kinds of tumour phenotypes, e.g. over – expression of CXCR4, Rac1, BMI1, and etc., in pancreatic tumour cell have observed a metastasis enhancement. In light of this, and hypothetically, in order to prevent metastasis, suppressing these targets should have a more pronounced effect in metastasis inhibition. Moreover, such outflanked approach may also prevent the tumour cell from switching into other signaling pathways producing the same tumour phenotypes, and achieving elimination ultimately. Besides, the current knowledge of each of these targets is insufficient, categorizing these targets by the tumour phenotypes produced and identify if there is any relationship between them and understand the

mechanism behind, would allow the discovery of linkages among them in terms of proteins and mRNA expression levels and functional activations.

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Single effort from one side is far from enough in pancreatic tumour elimination due to its high malignancy and complex tumour microenvironment, multiple targets have to be considered in developing PDAC therapies, therefore, the way of applying these targets and which targets should be applied require further effort."

3. Authors should elaborate more on the translational significance of each targets mentioned and commenting the targets with critical thoughts.

   Responses: In the revised version, we have tried to comment on each target based on its evidence for their anti – tumour activities which they are all promising in treating PDAC; speculated the relationships of several targets mentioned in the manuscript, e.g. TMPRSS4 is very likely to be related to E – Cadherin as an inverse expression pattern is observed between them; suggested to search for others alternatives to inhibit problematic targets, e.g. search for miRNAs that can inhibit both Rac1 and RhoA instead of targeting Rac1 alone; suggested other studies to answer the uncertainties in these targets, e.g. Notch signaling is found to be stage dependent in the regulation of pancreatic cancer development, thus studying the roles of Notch at each stage of pancreatic cancer should be able to help us understand more of the mechanism of Notch signaling.

   Again thank you very much for the Editors' effort in reviewing this manuscript, and your consideration will be highly appreciated.

Sincerely,

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