Revised MS for 86596

Original Title: Expression characteristics of peripheral Lymphocyte PD-1 and FoxP3+ Tregs in gastric cancer during surgery and chemotherapy

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Recently, tumor immunotherapy has become a field of advanced research with the advances of CAR-T-cells, genetically engineered T cells, CTLA-4, and the PD-1/PD-L pathway. However, the curative effects of tumor immunotherapy are often questioned, and the course of immunotherapy not fully understood. In this study, the authors aimed at evaluating the expression characteristics of peripheral Lymphocyte PD-1 and FoxP3+ Tregs in gastric cancer during surgery and chemotherapy to offer novel insights for future investigations into tumour immune evasion and the clinical application. The authors used primary clinical data, Flow cytometry analysis, and statistical analysis to verify their hypothesis. The results showed that significant increase of PD-1 expression on immune subsets and a larger number of FoxP3+ Tregs were observed in gastric cancer patients compared with healthy donors, which decreased after D2 gastrectomy notably. This phenomenon has never been observed before. So, in my opinion, this paper is well-written. The experiment design is reasonable, and the results reflects the conclusion as well. I recommend its acceptance after the minor revision. The detailed comments are: 1. In fig 1, we can see the significant increase of PD-1 expression on immune subsets and a larger number of FoxP3+ Tregs of patient compared with that of donors. And Fig 2 showed significant decrease of PD-1 expression on immune subsets of patients. I wonder what is the difference of PD-1 expression on immune subsets between patients and donors after D2 gastrectomy? 2. Several typo and grammar issues should be solved. For example, In the Flow cytometry analysis part, permeabilising should be permeabilizing.

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decrease of PD-1 expression on immune subsets of patients. I wonder what is the difference of PD-1 expression on immune subsets between patients and donors after D2 gastrectomy?

Answers:

Dear Reviewer,

Thank you for your valuable comments and insights.

In light of your feedback, we revisited the original data and conducted a comprehensive analysis on the PD-1 expression on immune subsets between donors and patients post-D2 gastrectomy. Upon re-evaluation, we found that the PD-1 expression in patients after D2 gastrectomy is indeed higher in most subsets compared to the donor control group.

Additionally, we further analyzed the difference in PD-1 expression on immune subsets between donors and patients after chemotherapy. Interestingly, our findings suggest that there is no statistically significant difference between the two groups.

difference of PD-1 expression between patients and donors after D2 gastrectomy

subsets	Donors (n=29))	*patients after D2* (n=29)	P
$PD\text{-}1^+$ cells	11.77±6.67	15.99±6.29	0.016
$CD4^+PD\text{-}1^+$	4.19±2.53	5.26±2.62	0.12
$CD8^+PD\text{-}1^+$	5.71±3.74	8.05±3.60	0.019
$CD45RO^+PD\text{-}1^+$	6.71±3.86	9.86±4.41	0.005
$CD4^+CD45RO^+PD\text{-}1^+$	2.67±1.57	3.67±1.80	0.028
$CD8^+CD45RO^+PD\text{-}1^+$	4.04±2.67	6.19±3.20	0.008

difference of PD-1 expression between patients and donors after chemistry

subsets	Donors (n=29))	*patients after chemistry* (n=29)	P
$PD\text{-}1^+$ cells	11.77±6.67	13.33±6.35	0.366
$CD4^+PD\text{-}1^+$	4.19±2.53	4.60±2.15	0.50
$CD8^+PD\text{-}1^+$	5.71±3.74	6.57±3.60	0.379
$CD45RO^+PD\text{-}1^+$	6.71±3.86	7.72±4.07	0.339

$CD4^+CD45RO^+PD\text{-}1^+$	2.67±1.57	3.28±1.73	0.168
$CD8^+CD45RO^+PD\text{-}1^+$	4.04±2.67	4.44±2.60	0.569

#2. Several typo and grammar issues should be solved. For example, In the Flow cytometry analysis part, permeabilising should be permeabilizing.

Answers:

We sincerely apologize for the oversight. Based on your feedback, we have reviewed the entire manuscript and have corrected the mentioned typo in the "Flow cytometry analysis" section from "permeabilising" to "permeabilizing".

Warm regards.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The authors used clinical data and samples to study the changes of peripheral Lymphocyte PD-1 and FoxP3+ Tregs in gastric cancer before and after surgery and/or chemotherapy. After reasonable compering the PD-1 expression and number of FoxP3+ Tregs in tumor patients and healthy donors, as well as at different stage during surgery and chemotherapy, the authors showed that the population of regulatory T cells was higher in the patients compared to the donors, which was similar to previous reports investigating prostate, lung, pancreatic and breast cancer. This result also provides a theoretical basis for the treatment of tumors with PD-1/PDL-1 blockers in combination with chemotherapy drugs. In short, the topic of this manuscript is timely and interesting. The authors have organized the manuscript rationally, with good methodology and well-written English. However, some important editing needs to be done before publication: 1) In this paper, one question has confused me. Why did the author simultaneously study the expression characteristics of peripheral Lymphocyte PD-1 and FoxP3+

Tregs in gastric cancer? What is internal connection of PD-1 and FoxP3+ Tregs? 2) The authors have provided detailed and accurate data on the changes of PD-1 expression and number of FoxP3+ Tregs in tumor patients at different stage during surgery and chemotherapy. So, what are or may be the novel insights for future investigations into tumour immune evasion and the clinical application of anti-PD-1 antibodies in gastric cancer? The author can daringly provide their perspectives.

#1) In this paper, one question has confused me. Why did the author simultaneously study the expression characteristics of peripheral Lymphocyte PD-1 and FoxP3+ Tregs in gastric cancer? What is internal connection of PD-1 and FoxP3+ Tregs?

Answers:

Dear Reviewer,

Thank you for your thorough review and insightful comments on our manuscript.

Addressing your query on why we simultaneously studied the expression characteristics of peripheral Lymphocyte PD-1 and FoxP3+ Tregs in gastric cancer: The choice was grounded in the emerging understanding of the intertwined roles these components play in the tumor immune microenvironment. As you rightly mentioned, the relationship between the PD-1/PD-L1 pathway and Tregs remains intricate and is yet to be fully understood.

The involvement of Tregs in the treatment of PD-1/PD-L1 blockade and the influence of the PD-1/PD-L1 axis on Treg differentiation and function have been increasingly emphasized in recent studies. This accentuates the need to understand their coexistence and potential interactions, especially in the context of gastric cancer.

With respect to peripheral PD-1 expression, while it has been documented in gastric cancer patients, the sequential evaluation from pre-surgery to post-chemotherapy stages remains largely uncharted territory Our attempt was to bridge this knowledge gap and to additionally shed light on the relationship between peripheral PD-1 expression and FoxP3+ Tregs in these patients – an area where extant literature is noticeably sparse.

2) The authors have provided detailed and accurate data on the changes of PD-1 expression and

number of FoxP3+ Tregs in tumor patients at different stage during surgery and chemotherapy. So, what are or may be the novel insights for future investigations into tumour immune evasion and the clinical application of anti-PD-1 antibodies in gastric cancer?

Answers:

Dear Reviewer,

Thank you for your constructive feedback and for highlighting the significance of the insights our study may offer for future investigations.

In response to your inquiry regarding the potential novel insights our research could offer in the realm of tumor immune evasion and the clinical application of anti-PD-1 antibodies in gastric cancer:

1.Tumor Immune Evasion Mechanisms: Our study underscores the dynamic nature of PD-1 expression and FoxP3+ Tregs number in patients at various stages of treatment. This dynamism hints at the potential adaptive strategies tumors might employ to resist immune attack. Specifically, the interplay between FoxP3+ Tregs and the PD-1/PD-L1 pathway might be a significant player in these evasion mechanisms. Understanding this relationship in depth could pave the way for interventions to counteract these mechanisms and restore anti-tumor immunity.

2.Clinical Application of Anti-PD-1 Antibodies in Gastric Cancer: The nuanced understanding of PD-1 expression patterns throughout treatment stages could provide a blueprint for optimizing the timing and dosing of anti-PD-1 therapies. There is potential for combination therapies, where modulating the number or function of FoxP3+ Tregs can potentiate the efficacy of anti-PD-1 antibodies, offering a synergistic approach.

3.Predictive Biomarkers: Our findings might also hint at the role of peripheral PD-1 expression and FoxP3+ Tregs levels as predictive biomarkers.

We appreciate your encouragement to "daringly provide our perspectives". We indeed believe that a deeper dive into the confluence of PD-1 expression and FoxP3+ Tregs in the context of gastric cancer could usher in innovative therapeutic strategies and improved patient outcomes. We will enhance our discussion section to include these perspectives more prominently.

Warm regards.