

Peer Review File

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Reviewer

The manuscript entitled ‘Primary tumour immune score fails to predict the prognosis of colorectal cancer liver metastases after hepatectomy in Chinese populations’ researched the prognostic role of primary tumour immune score in colorectal cancer liver metastases (CRLM) after hepatectomy in Chinese patients, by stating whether the immune score of primary tumour could predict the prognosis of liver metastases after hepatectomy in Chinese patients.

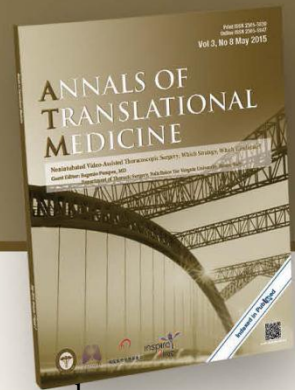
1. In this paper, it is pointed out that the primary tumor and the corresponding metastatic tumor have different immune environment. What are the specific differences?

Reply 1: Our observations show that CD3+ and CD8+ lymphocytes infiltration in the primary tumor is higher than the corresponding liver metastasis. Immune score of the liver metastases correlates with the post-hepatectomy OS while those of the primary tumor are not related to the prognosis. However, we did not analyze the overall immune signatures of the immune microenvironment in primary tumor and liver metastasis. Therefore, the use of the term “immune heterogeneity” may not be appropriate in the manuscript. We have modified the main text to make the narration more precise.

Changes in the text: We modified the main text to avoid the use of “immune heterogeneity”.

2. What are the underlying mechanisms of immune heterogeneity between primary tumor and liver metastasis?

Reply 2: Thank you for your comment. As it is stated in the Discussion section, the underlying mechanism of immune heterogeneity between primary tumor and liver metastasis remains unclear. However, we have provided several hypotheses. Firstly, patients who received



preoperative chemotherapy during the interval between primary tumor resection and hepatectomy may have an altered immune microenvironment in the metastases that is different from the primary tumor. Notably, in patients who received synchronous resection or received no pre-hepatectomy chemotherapy, the immune infiltration between primary tumor and liver metastasis shows no significant correlation, either, implying that this is not the only potential mechanism. Another potential mechanism may be that liver metastases originate from different malignant clones of primary tumor with different genetic and epigenetic alterations and accumulate mutations during the metastatic process, which may cause the immune heterogeneity between primary tumor and liver metastases. Finally, the inherent immune microenvironments of primary colorectal cancer and liver metastases are different and react differently to the tumor cells, resulting in different immune infiltration.

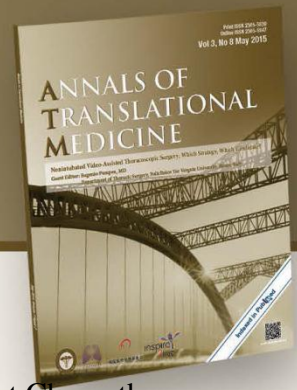
However, the present study did no further research on the underlying mechanism of immune heterogeneity, therefore, future study is needed to verified the hypothesis mentioned above.

Changes in the text: We have modified our text in the Discussion section, Line 396-423.

3. In the case of heterochronous resection and hepatectomy chemotherapy between primary tumor resection and hepatectomy, liver metastasis may have a different immune pattern from that of primary tumor. There is a hypothesis that chemotherapy may change the immune microenvironment. Is there any other hypothesis to support this theory?

Reply 3: It is under debate that whether the change of immune microenvironment is a cause or a subsequence of preoperative chemotherapy. However, hypotheses about the effect of chemotherapy have on immune microenvironment have been provided.

One hypothesis provided by Van den Eynde M. suggested that the cytotoxic effect of preoperative chemotherapy causes the death of tumor cells, which could release antigen to further enhance the anti-tumor immune infiltration (Van den Eynde M. et al. The Link between the Multiverse of Immune Microenvironments in Metastases and the Survival of Colorectal Cancer Patients. *Cancer Cell*. 2018;34(6):1012-26 e3). This hypothesis is supported by study on breast cancer revealing that neoadjuvant chemotherapy enhances immune infiltration in microenvironment and reflects prognosis (Anne-Sophie Hamy, H el ene Bonsang-Kitzis, Diane De Croze, et al. Interaction between Molecular Subtypes and Stromal Immune Infiltration



before and after Treatment in Breast Cancer Patients Treated with Neoadjuvant Chemotherapy. Clin Cancer Res. 2019 Nov 15;25(22):6731-6741.). Our observation also supports the mentioned hypothesis and finds that both CD3+ and CD8+ lymphocytes densities in the CT and IM area are higher in cases that received preoperative chemotherapy.

However, both our research and reports of the mentioned studies did not analyze the alteration of tumor microenvironment before and after the pre-hepatectomy chemotherapy within the same patient or malignant lesion. Therefore, self-control researches that compare the immune microenvironment before and after preoperative chemotherapy are needed.

Changes in the text: We have modified our text in the Discussion section, Line 403-408.

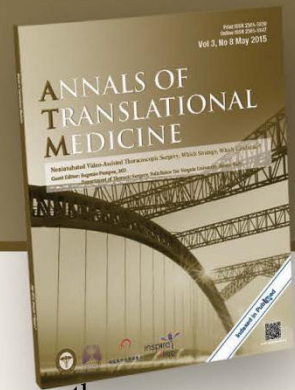
4.Heterogeneous immune pattern was observed between primary tumor and liver metastasis. It may be that metastatic tumor cells originated from different malignant clones of primary tumor. Is there any evidence to support this theory?

Reply 4: Thank you for your question. Phylogenetic reconstruction of tumor revealed that heterogeneity exist within the same lesion(Gerlinger, M. et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N. Engl. J. Med. 2015;366, 883–892.). Previous study has reported that immune heterogeneity exists among multiple metastases within the same patient (Van den Eynde M. et al. The Link between the Multiverse of Immune Microenvironments in Metastases and the Survival of Colorectal Cancer Patients. Cancer Cell. 2018;34(6):1012-26 e3). Therefore, we provided the hypothesis that the different immune status between primary and metastatic lesion may attribute to that metastatic tumor cells derive from different malignant clones of primary tumor and accumulate genetic mutations during the metastasis process. Phylogenetic analysis about the origination of metastatic tumor cells is needed to further verify the hypothesis.

Changes in the text: We have modified our text in the Discussion section, Line 411-416.

5.Is there any difference in immune score of primary tumor among different pathological types of rectal tumor?

Reply 5: A supplemental analysis is conducted to present the differences in immune score of primary tumors among different pathological types of rectal cancer. Our observation shows that



immune scores of rectal mucinous carcinomas tends to have a lower immune score than non-mucinous carcinoma but the difference is not significant. Notably, of 47 rectal cancer cases, only 4 cases are pathologically diagnosed as mucinous carcinoma. Therefore, further study focusing on immune microenvironment in different pathological types of rectal cancer is needed to certify the difference. However, the present study classified primary colorectal tumor location into right-sided and left-sided and carried out the subsequent analyses, therefore we did not analyze the difference among different pathological types of rectal tumor separately in the main text.

Changes in the text: We did not modify the main text but we would like to provide a supplementary table to answer the question.

Supplementary Table Immune score of primary rectal cancer with different pathological types.

Immune score	Pathological types of primary rectal cancer		<i>P</i> value
	Mucinous adenocarcinoma	Ductal adenocarcinoma	
0	1	11	0.429
1	2	7	
2	0	7	
3	1	8	
4	0	10	
Median immune score	1	2	
Low ^a	3	26	0.638
High ^b	1	18	

a: Immune score 0 to 2 is defined as low immune score. b: Immune score 3 to 4 is defined as high immune score.