Peer Review File

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Reviewer A

Tumor details are not revealed, more markers, tumor stage, tumor cell content. Interesting method to kill mice by unseparated MPE cells. Interesting approach to inject undefined junk cell populations into immunecompromized mice. The regular method is: cultivate NSCLC cells in vitro, tumor cells attach within a short time, other cell populations can be removed, tumor cells are harvested and counted and a defined population is used for establishment of PDX. Here, the constitution of the the MPE-derived bulk population is not known (maybe avoid of tumor cells?) and the NSCLC markers of all successful samples is not known. Thus, the data are not usable. Chemosensitivity can be tested after first in vitro passages, at advanced stages of NSCLC MPE may consist of almost pure tumor populations. Why MPE should be necessary for patients is beyond my imagination, the term "un-necessary" instead of impairing breathing is strange.

Reply:

First, we really appreciate the reviewer for taking time to read our manuscript and providing useful comments. In accordance with the reviewer, we added tumor stage, representative tumor markers CYFRA and CEA, and timing of collection of MPE in Table 1. Note that all cases are stage IV when tumor cells are detected in pleural effusion, so the stage at the time of lung cancer diagnosis is noted. Tumor cell content in MPE was not examined.

We describe the MPE management and inoculation method as limitations of this study. As reviewer pointed out, we injected unselected cell populations into mice. We chose the simplest possible method for inoculating MPE-derived cells. We took into consideration the fact that it is not easy to completely remove all non-tumor cells in a short culture period, that some tumor cells in pleural effusion have a tendency to float, and that we wanted to reduce the amount of labor involved. We added this information in the revised manuscript.

The mechanisms by which mice die by inoculation with MPE-derived cells is not well understood, but findings consistent with GVHD were observed. The details of the origin of inflammatory cell infiltration consistent with GVHD have not been evaluated as noted in limitation.

At just after inoculation with MPE-derived cells in mice, tumor formation is unrecognizable. After a few weeks, tumor formation became recognizable, and the tumor subsequently increased over time. Pathological confirmation that the masses formed in mice were cancerous was obtained in all cases. Special staining of tissue samples with anti-human antibodies was not performed because it was deemed unnecessary for the diagnosis of cancer. Tumor markers in the blood of mice were not measured.

As the reviewer suggested, chemosensitivity can be tested using with MPE. We are planning to conduct chemosensitivity test. However, that is outside the scope of this study and is not included in this study.

We deleted the term “unnecessary” according to the reviewer.
Thank you very much again for all the advice.

Changes in the text:
(Abstract: line 36) “unnecessary for patients” was changed to “discarded”.
(Highlight box: line 57) “unnecessary for patients” was changed to “discarded”.
(Introduction: line 66) “Excessive MPE is generally unnecessary for patients” was changed to “excessive MPEs are usually discarded”
(Results: lines 167-170) At just after inoculation with MPE-derived cells, mass formation is unrecognizable. The subsequent process of tumorigenesis varied from case to case, with the fastest case becoming recognizable after 2 weeks and the slowest after 12 weeks after inoculation. The tumor subsequently increased over time.
(Discussion: lines 297-301) We injected unselected cell populations into mice. We chose the simplest possible method for inoculating MPE-derived cells. We took into consideration the fact that it is not easy to completely remove all non-tumor cells in a short culture period, that some tumor cells in pleural effusion have a tendency to float, and that we wanted to reduce the amount of labor involved.
(Figs 2 and 3) Figs 2G and 3E were added.
(Table 1): We added tumor stage, representative tumor markers CYFRA and CEA, and timing of collection of MPE.

Reviewer B

This study aimed to establish cell lines and generate tumor using mouse models by malignant pleural effusion (MPE). This paper could serve as important foundational data for cell lines and animal experiments. However, several adjustments need to be made to satisfy scientific evidence.

In Fig 1, established cell line formations, such as 16-03, 16-10, and 17-05, should be presented to consider morphological changes or differences in cell lines like as 15-01, and 15-05.

Reply: First of all, we really appreciate the reviewer for careful reading and useful comments to improve our manuscript. Unfortunately, we did not take photographs of primary cell conditions of all MPEs. We have photographs of primary state of PE17-05 but not PE16-03 and PE16-10. These have been established as cell lines and are stored in liquid nitrogen. Therefore, it is possible to take photographs of the cell line state by taking them out and culturing them. However, since we can never photograph the primary state of PE16-03 and PE16-10, we are unable to present them for comparison.

Changes in the text: (no changes)

How cell line selected for use in mouse xenograft? Please specify which cell line was inoculated. if only two animals were experimented on, it would pose difficulties for scientific validation.

A minimum of five animals per group is necessary for adequate statistical significance and to confirm statistical validity.

Reply: We performed mouse xenograft experiments using with all 28 MPE samples, but not established cell lines. The number of mice used was two per each MPE sample. The small number of mice used could have resulted in inaccurate results, as noted by the reviewer. For examples, in PE17-05, tumorigenesis was observed in only NOD-scid, but not in NSG. If multiple NSG mice had been used, tumorigenesis might have been observed. Although tumorigenesis was observed in 6 of 28 cases, it is conceivable that this probability would increase if more mice were used. Even though tumorigenesis in individual cases may be imprecise, it is true that there are cases of successful tumorigenesis. However, the small number of mice used per each case was added to the revised manuscript as a limitation of this study.

Changes in the text: (Discussion: lines 303-306) Fourth, the number of mice used was two per each MPE sample. The small number of mice could have resulted in inaccurate results. Although tumorigenesis was observed in 6 of 28 cases, it is conceivable that this probability would increase if more mice were used.

During mouse xenograft monitoring, were there any changes in tumor size by each MPE? If there were changes of tumor size by MPE, it could be significant results.

Reply: Of course, tumor sizes change during monitoring. At just after inoculation with MPE-derived cells, mass formation is unrecognizable. The subsequent process of tumorigenesis varied from case to case, with the fastest case becoming recognizable after 2 weeks and the slowest after 12 weeks after inoculation. The tumor subsequently increased over time. We added this information in the revised manuscript, and the time courses of tumor growth for PE15-05 and PE17-08 were added as Figs 2G and 3E, respectively.

Changes in the text: (Results: lines 167-170) At just after inoculation with MPE-derived cells, mass formation is unrecognizable. The subsequent process of tumorigenesis varied from case to case, with the fastest case becoming recognizable after 2 weeks and the slowest after 12 weeks after inoculation. The tumor subsequently increased over time. (Figs 2 and 3) Figs 2G and 3E were added.