Author's response to reviews

Title: Molecular characteristics of endometrial cancer coexisted with peritoneal malignant mesothelioma: a case report of Li-Fraumeni-like syndrome

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Author's response to reviews: see over
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Dear Editor:

On behalf of my colleagues, I am resubmitting the revised manuscript #R1-3459800851385705, entitled "Molecular characteristics of endometrial cancer coexisting with peritoneal malignant mesothelioma in Li-Fraumeni-like syndrome" for your consideration for publication as a “Case Report” in BMC Cancer. I assume full responsibility that all authors have agreed to be so listed and have seen and approved the manuscript, its content, and its submission.

First of all, we are grateful for reviewers’ positive comments and specific advices. Upon the reviewer's request, we have added three files of Supplementary Information (SI: 1 to 3). Furthermore, we reanalyzed the somatic mutation results derived from the data of OncoScan MIP microarrays according to the OncoScan analysis algorithm for somatic mutations, which is described in Supplementary Information 3. Using the stringent criteria, we ruled out the 4 mutations that were listed in the previous Table 1. The 4 mutations are TP53 (844C>T), TP53 (524 G>A), KRAS (35G>C), and PTEN (388C>G). We further confirmed the absence of TP53 (844C>T) in all specimens of this case using another sensitive platform- digital PCR. Therefore, the revised contents of Table 1 are less than the previous version.

Reviewers’ suggestions are replied, point-by-point, as follows. Revisions are marked in red in the revised manuscript.

Reviewer: Chen Wang
Reviewer's report: I read this manuscript with great interest. However, English presentation and a lot of clarifications are needed to make it acceptable.

Reply: English editing is performed by Dr. Shihtien Wang (Assistant Professor, Children’s Hospital of the University of IL Med. Ctr., Chicago, IL 60612). Specific clarifications are described as follows.

• Major Compulsory Revisions
1. Sequencing and mutation-calling methods need to be described. I understand it’s a case report, but some supplementary information will be helpful for who are interested to learn details. For examples, “TruSeq Amplicon Cancer Panel” contains how many genes/exons? The whole-gene body or just selected exons or some mutation hotspot regions were sequencing? Similarly, how many probe-sets the Affymetrix Oncoscan molecular inversion probe microarray has? How exactly the CNV was called? I’m also surprised by very low resolution of CNV plots in Fig. 2.

**Reply:**

Three files of Supplementary Information (SI) are added. SI-1 lists detailed information of TruSeq Amplicon Cancer Panel. SI-2 describes how copy number variations are called with OncoScan MIP microarrays and lists the chromosomal regions that are covered by 217,611 probe sets on OncoScan microarray. SI-3 describes the algorithm of interpreting somatic mutations with OncoScan MIP microarrays and lists the detailed information on nature of the 76 somatic mutations. A new reference #9 is added in the revised manuscript to describe the details of molecular inversion probe microarray.

To demonstrate the resolution of CNV plots, two Figures are added as Figures 2B and 2C, where selected regions of chromosomes 10 and 19 are zoomed in (lines 284-287 on p. 17).

2. I’d suggest authors adding some extended discussion on Li-Fraumeni-like syndrome. How prevalent is? What’s the implication of this case study? Would authors suggest doing mutation panel for all new patients diagnosed? Or, for a selected subset of patients, with what criteria?

**Reply:**

Two revised paragraphs in Discussion (lines 158 to 173, pp.15-16) are devoted to discuss these important points that the reviewer raised. To this extent, two new references, #13 and #20 are added in the revised manuscript.

3. More clinical information is needed for me to digest this patient case. Tumor grade of endometrial cancer? Histology? How successfully the surgery was done, i.e. residual disease/debulking status? It’s unusual to see a late stage (IIIC) patient with good response to chemo after 24 months.
**Reply:**
The requested information is written in lines 69 to 76 on p.6 of the revised manuscript. The patient received both chemotherapy and radiotherapy after the surgery (lines 71-78 on p. 4). In our institution, the 5-year-overall survival for stage IIIIC endometrial cancer is 75%, and therefore a new reference # 7 is added.

4. abbreviations sometimes were used without full names spelled out. (CEA, ER, PR, Ber-EP4), sometimes redundantly explained multiple times (e.g. Em Ca = endometrial cancer). The authors should keep certain consistency, either spelling out at 1st time or explaining all of them in the abbreviation appendix.

**Reply:**
This type of errors (p.4) is corrected according to the reviewer's advice on page 4.

5. inconsistent and confusing usages of “plasma” and “blood” in the 1st page. “plasma DNA of the patient were sequenced using targeted …”, and then followed by “TP53 mutations in blood”. Plasma DNA is degraded and germline (blood) is usually of much longer fragment size. Please clarify.

**Reply:**
All "plasma" words are changed to "blood". Thank you.

• Minor Essential Revisions
1. CNV presentation could be more informative in fig.2, such as using height of the bar to indicate amplitude of copy number changes?

**Reply:**
In this case, all CNV alterations were only 1 copy difference, i.e., amplified to become 3 copies or deleted to 1 copy. Figures 2B and 2C are added to show the amplitude of copy number changes in selected chromosomal regions.

2. I’d appreciate more details about PMM/endo comparison. To confirm it’s not metastasis, rather, it’s co-occurred tumors at different places. For example, is there some common genomic abnormalities indicative likely common origin of different tumors?

**Reply:**
The genomic changes of PMM and endometrial cancer in this case are very different, as depicted in Figure 2 and Table 1. The only mutation in common of two cancers in this case was the germline mutation of TP53 indicating Li-Fraumeni-like syndrome, which may explain multiple primary cancers in a patient.

3. **table 1 is not interpretable. Are these column names even comparable? Em Ca and PMM are tumors; Miseq and OncoScan MIP microarray are measuring techniques, and even Miseq is not comparable to OncoScan platform, since authors mentioned it’s some Truseq amplicon panel.**

**Reply:**
We have re-organized the presentation of Table 1 with new results.

**Reviewer:** Steven Hart

**Reviewer's report:**

Minor Essential Revisions
Page 4 Line 76. The authors refer to the low grade mesothelioma, but should also include what pathological features triggered the followup staining for CK5/6 and Cer-EP4

**Reply:**
Ber-EP4 is positive in adenocarcinoma, but negative in mesothelial cells. CK5/6 is often positive in mesothelial cells and mesothelial tumors, but negative in adenocarcinoma. These two immunostaining were done to confirm the diagnosis of mesothelioma and exclude metastatic adenocarcinoma. The information is added on lines 78-84, p. 4.

Discretionary Revisions
Table 1. should also contain the tumor % so as not to misinterpret negative findings as an issue with analytic sensitivity. This could simply be done for the MiSeq results where the number of reference and alternate alleles are give.

**Reply:**
The tumor % and more information are added to the revised Table 1.

I would strongly encourage the authors to include a supplement detailing all of the chromosomal coordinates for amplifications and deletions so that the next time one
occurs, this will be available.

Reply:

Three files of Supplementary Information (SI) are added. SI-1 lists detailed information of TruSeq Amplicon Cancer Panel. SI-2 describes how copy number variations are called with OncoScan MIP microarrays and lists the chromosomal regions that are covered by 217,611 probe sets on OncoScan microarray. SI-3 describes the algorithm of interpreting somatic mutations with OncoScan MIP microarrays and lists the detailed information on nature of the 76 somatic mutations.

Thank you very much for the kind consideration of our paper.

Yours sincerely,

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