Title: Pseudomyxoma peritonei - two novel orthotopic mouse models portray the PMCA-I histopathologic subtype

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

Please find responses to comments from the reviewers in this letter as well as the accordingly revised manuscript entitled “Pseudomyxoma peritonei – two novel orthotopic mouse models portray the PMCA-I histopathologic subtype”. Below (pp 2-4), each comment has been addressed in detail. We hope you find our responses and suggestions for revising the manuscript satisfactory and look forward to hearing from you.

Sincerely,

Kjersti Flatmark
MD, PhD
Reviewer 1

Major Compulsory Revisions

Comment:
My major criticism is personal but I think important. My colleagues and I have published several papers in which we claim (and I think demonstrate) that all forms of PMP are malignant. Thus, we do not accept DPAM in the Ronnett classification.

References include: Bradley et al Am J Surg Pathol 2006;30:551-9 and Stewart et al Ann Surg Oncol 2006; 13:624-34. We categorize PMP as 2 entities: mucinous carcinoma peritonei (MCP), low grade and MCP, high grade. The malignant nature of DPAM was shown more recently (Geisinger Am J Clin Pathol 2007; 135-43) as several cases metastasized to the pulmonary parenchyma. I believe the authors should acknowledge this new classification in the introduction and discussion. (From the description of their 2 models, it appears as if they would be considered by us as MCP, low grade).

Response:
We realize that the classification of pseudomyxoma peritonei is somewhat controversial, but we had not intended to address this in the manuscript. To acknowledge the existence of an alternative classification, we have rephrased the last sentence in the top paragraph at page 4 of the introduction, with reference to Bradley et al, Am J Surg Pathol 2006. However, since the establishment of animal models was the main topic of this manuscript and not classification of pseudomyxoma peritonei we have not included this subject in the discussion section.

Comment:
In the last paragraph of the Introduction, the authors state that PMP is characterized by noninvasive growth. This is true in the majority of instances, but does occur in a large minority. This is stated in the histologic classification of Ronnett that they cite. This needs to be addressed.

Response:
We agree that this issue was presented in a somewhat simplified manner, and to alleviate this, the statement has been removed.

Comment:
Did either or both patients receive chemotherapy before tissue was taken for growth in mice? This could affect some aspects of the behavior or characterization of the tumors. This should be stated in the Material section.

Response:
No chemotherapy was given to either of the patients prior to implantation in animal models. One sentence stating this fact has been added at page 5 at the end of the introductory paragraph in the section “Patients”.

Comment:
Patient #2 had a primary adenocarcinoma, but in the model most of the tumor cells appeared benign (adenomatous). Can they please explain that in the results or discussion section(s)?

Response:
This finding surprised us when the primary sections were reassessed. The primary lesion was classified a mucinous adenocarcinoma because of a small area of the tumor exhibiting invasive growth. Neither in the second lesion in the left ovary nor in any section from the main surgical specimen was invasive growth observed. This is certainly an interesting observation, but one that we cannot explain.

Discretionary Revisions

Comment:
In the second paragraph of the discussion the authors state that one difference between human and mouse tumor is the free, fluid based growth of the latter by cells in the murine peritoneal cavity. Perhaps this helps to explain the lack of invasion of tissue in their model.

Response:
This might of course be part of the explanation, but we actually see this growth pattern in humans quite often, but usually in combination with “solid” tumor tissue that adheres tightly to the peritoneum and intraperitoneal organs.
Reviewer 2

Major Compulsory Revisions

Comment:
1. Number of mice studied and the reproducibility of the model: it is not clear from the abstract, the paper text or the tables what was the number of mice studied. What was the rate of tumor uptake? Why were mice injected sc? What were the corresponding results in contrast to the ip group? My major concern relates to the reproducibility of the model – were the authors able to freeze tumor cells generated in the model, then thaw them and inject again?

Response:
This point contains several comments:
- **Number of mice studied and the reproducibility of the model: it is not clear from the abstract, the paper text or the tables what was the number of mice studied.**
  As to the number of mice studied, this was regretfully left out. In the methods section at the end of the “Tumor models” paragraph, a phrase has been added to specify the number of mice implanted or injected per passage. At page 8 in the results section a sentence has been rephrased to emphasize that both i.p. models had reached passage 14 whereas the s.c. PMP1 model was in passage 5.
- **What was the rate of tumor uptake?**
  In the results section page 8 we have specified that the take rate was close to 100%.
- **Why were mice injected sc? What were the corresponding results in contrast to the ip group?**
  Mice were not injected s.c., but tumor pieces were implanted, successfully in the PMP1 model, but not for PMP2. Why we performed s.c. grafting? Well, initially because we had never tried to grow these cells in vivo before, and did not know that the i.p. strategy would be the more successful. Tumor take s.c. was 0 in PMP2, and close to 100% in PMP1, but with tumors growing much more slowly than i.p., illustrated by the respective passage number at the preparation of the manuscript mentioned in the results section page 8.
- **My major concern relates to the reproducibility of the model – were the authors able to freeze tumor cells generated in the model, then thaw them and inject again?**
  As specified in the results section page 8 we were able to reestablish growth from harvested tissue stored at -196°C for 3-6 months. This was performed at least twice for each model.

Comment:
2. Relevance: what would be the parameters to be used in measuring effect of drugs in this model – the tumor burden? Amount of ascites? The authors have to demonstrate the reproducibility of this factor/s.

Response:
Although we think our models will be useful, for instance to assess drug efficacy in vivo, we do not claim that the models have been fully validated for this purpose yet. We shall probably try to use the volume or weight of mucinous tumor tissue as a therapeutic end point. We entirely agree with the reviewer that this must be properly validated when and if the models are to be used for this purpose, but this is somewhat beyond the scope of the present study.

Comment:
3. In the section of methods on IHC, page 7: positive cells defined per field? How many areas analyzed per specimen?

Response:
Specifications of these details have been added in the methods section page 7.

Comment:
4. Present the limitations of the model in the section of discussion.

Response:
The limitations of the models, apart from the obvious limitations of animal models portraying human disease, have not been comprehensively addressed in this study, and we cannot accurately conclude as to what will be the limitations of the models at the present time.
Minor Essential Revisions

Comments:
1. Typo in the first line of methods page 2: “in the two peritoneal cavity”.
2. Typo in 3rd line, section methods/patients page 4: the word obtaining is missing (to obtain an informed consent)
3. Typo page 8: “been stored at”...
4. Table 1: list of positive controls is not full.

Responses:
1. Has been corrected
2. Has been corrected
3. Has been corrected
4. Intestinal mucosa and colon cancer served as positive controls for all three antibodies. Since this may be misunderstood, we suggest a brace be inserted to underline this, unless this will be a problem with the journal layout (see table 1).