Author’s response to reviews

Title: Metalloproteases meprin-ɑ (MEP1A) is a prognostic biomarker and promotes proliferation and invasion of colorectal cancer

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Author’s response to reviews:

Dear Editor:

Thank you for your kind letter of “Your submission to BMC Cancer - BCAN-D-16-00008R1” on 21 Jan 2016. We revised the manuscript in accordance with the reviewers’ comments, and carefully proof-read the manuscript to minimize typographical, grammatical, and orthographical errors. Here below is our description on revision according to the reviewers’ comments.

Reviewer 1

Major comments:

1. The quality of written English is insufficient. A complete grammatical and orthographical revision of the manuscript is recommended. In this way some statements and descriptions in material/methods and results section are very difficult to follow when reading the manuscript the first time.
2. The authors compared mRNA and protein expression of MEP1A in 36 CRC patients with paired normal controls. In my understanding this cohort is different from the 88 CRC patients which were analyzed in high and low MEP1A expression group and correlated with clinical scores and survival (and this group had no control cohort!). Why did the authors study a second group of CRC patients? Which criteria were used for matching with healthy controls (age and gender?)? For this cohort clinical data (tumor growth, N and M stage and differentiation) are needed since the authors conclude a positive correlation for these parameters in the 88 CRC patients group.

This issue with 2 different cohorts is very confusing and difficult to understand and must be clarified. Maybe it would be helpful to rearrange the material/methods and results section.

3. It would be interesting if it is possible to determine a cutoff for the MEP1A mRNA expression.

As mentioned by the authors, only patients who had undergone tumor resection without chemotherapy or radiotherapy were included in this study. This important point might be a potential selection bias. Further data regarding patients with advanced and palliative disease should be included to correlation and survival analysis. Otherwise this limitation has to be clearly stated in the discussion which completely is lacking limitations of this study.

4. The laboratory results regarding effect of MEP1A attenuation to proliferation and invasion in vitro and in vivo are promising. To my point of view the results of the authors would be strengthen by another experimental setting regarding MEP1A silencing and overexpression. In this way overexpression of MEP1A should lead to elevated proliferation, invasion and migration compared to control. Alternatively, the authors could compare Caco2 or LoVA cells (high MEP1A expression) with NCM460 cells (low MEP1A expression). These analyses would further support the findings of the authors.

5. In vitro analyses regarding E-cadherin silencing and overexpression could lead to further insights relating to clinical findings of MEP1A/E-cadherin association.
Minor comments:

1. The abstract section of p2 is not congruent with title page, especially results paragraph is very different and therefore confusing.

2. The third paragraph of introduction has no relation to the rest of the manuscript and is dispensable to my point of view.

3. In materials and methods cell culture is described without antibiotics. Were P/S used for cell culturing?

Answers to major comments:

1. The spelling and syntax errors have been checked and corrected.

2. The reasons why we studied two groups of CRC patients are as follow: First, the two groups’ form were different, the 36 patients group consisted of 36 paired tissue specimens (each specimen’s volume is 0.5cm*0.5cm*0.5cm) and the 88 patients group consisted of two tissue microarrays (88 paired specimen points on each slide, one slide used to detect MEP1A, the other one used to detect E-cadherin). Second, the 36 CRC patients group had no follow-up data since these specimens were collected only almost one year ago (between October 2014 and April 2015), so we only used this group to compare mRNA and protein expression of MEP1A. Third, the 88 patients group had an over 5 years’ follow-up data and a detailed clinico-pathological data, so we used this group to determine whether MEP1A expression correlated with CRC clinico-pathological factors and survival.

The reviewer 1 said “this group had no control cohort”. In fact, the 88 patients group had a control cohort, because they are paired specimen points, each tumor specimen point had a paired adjacent normal mucosal specimen point on its right side.
3. Now, we have listed some limitations of our study in the last paragraph of discussion. In the future, some in-depth studies are needed to investigate whether patients with advanced and palliative disease have a different results in correlation and survival analysis.

4. Due to time constraints, MEP1A overexpressing cell lines were not investigated in our study. We planned to conduct the overexpression experiments in the next phase of the study.

5. Your suggestion is very instructive, we will consider to add some E-cadherin silencing or overexpression experiments in the future study.

Answers to minor comments:

1. The P2 of the abstract section has been re-organized and it is congruent with the results section now.

2. The third paragraph of introduction has been deleted.

3. It is our negligence that we had forgotten to describe the use of antibiotics in cell culture. It has been corrected now.

Reviewer 2

Minor comments:

1. Abstract

Page 2 Line 9: The first sentence in the methods section is clumsy and could do with rewriting.
2. Introduction

P3 L11: Suggest change to 'in CRC development and progression. '

P3 L52-54: Provide reference(s) and correct 'additionally, human prostate cancer cell models also express MEP1A, where it promotes cellular replication and invasiveness (delete 'in these cells').'

3. Methods

P4 L19: Please clarify the number of CRC patients. N=36 here. Then n=40 on P5 L37.

P4 L29-32: Suggest change to 'Two pathologists confirmed the diagnoses and performed tumor staging according to the guidelines of the…'

P6 L1: Suggest change to 'protocol used the following conditions: '

P7 L42: Correct 'describede'.

4. Results

P10 L1-2: Rewrite 'The mean MEP1A mRNA expression found in tumor tissue specimens and in paired adjacent normal mucosal specimens was significant'. State how it was significantly different.

P10 L13: Correct 'suggests'.

P10 L29: Provide p values for PCNA and Ki-67 mRNA expression differences.
P10 L46: Clarify that migration and invasion assays were only performed in LoVo cells. Either change the subtitle to 'Decreased LoVo cell migration...' or change the first sentence to '..., scratch and migration invasion assays were performed using LoVo cells (Figure 4A and B).'

P11 L27-39: Move Table 1 and 2 under the subheading 'Correlation between MEP1A overexpression and clinicopathological factors in CRC'.

P12 L15: Delete 'Table 3 (in page 21)'.

P12 L35: Which factors were taken into account by the multivariate analysis?

5. Discussion

P12 L52: Delete the first 'both' in 'both expression of both MEP1a..'

6. Figures and Legends

Expand Table 1 Legend to state that these are the n=88 patient samples on the Tissue Microarray.

Figure 1. Correct 'Graycale' on Y axes.

Figure 5B. Use 'Cumulative Survival' on Y axis.

Answers to minor comments:

1. All spelling and syntax errors have been checked and corrected.

2. As shown in Table 3, factors which were taken into account by the multivariate analysis were tumor location, AJCC stage, LNM stage, distant metastasis, vascular invasion and the expression of MEP1A and E-cadherin.

Thank you and all the reviewers for the kind advice.
Sincerely yours,

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