Author’s response to reviews

Title: Endothelial Cell-Specific Molecule-1 as an Invasiveness Marker for Pituitary Null Cell Adenoma

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Author’s response to reviews:

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Dr. Zuleyha Karaca
BMC Endocrine Disorders
https://bmcendocrdisord.biomedcentral.com/

Re: Ms. Ref. No.: BEND-D-19-00130

Dear Dr. Zuleyha Karaca,

We thank you for the opportunity to revise our manuscript, entitled “Endothelial Cell-Specific Molecule-1 as an Invasiveness Marker for Pituitary Null Cell Adenoma”.

Detailed point-by-point replies to the reviewers’ comments in addition to our revised manuscript have been resubmitted. All changes made in the revised manuscript were indicated using the track function of WORD in the revised manuscript.

We hope our revised manuscript could be acceptable for publication in BMC Endocrine Disorders. We thank you once more for your time and consideration.

Sincerely,

Shousen Wang
Reviewer reports:

Takatoshi Anno, MD, PhD (Reviewer 1): In this manuscript, the authors reported it is possible that ESM-1 expression would become an invasiveness marker for pituitary NCA. In NCA, ESM-1 expression was associated with tumor invasion and tumor size in vascular endothelial tissues but only tumor size in adenoma cells, thus concluding that pituitary NCA progression occurs through ESM-1 associated angiogenesis. However, this report includes several problems that should be addressed.

1. For new WHO classification of pituitary adenoma, "null cell adenoma requires the demonstration of immunonegativity for pituitary transcription factors and adenohypophyseal hormones". The authors are necessary to use 4th edition of the WHO classification even if the data of sample collection was before 2017.

In addition, "the term of atypical pituitary adenoma is no longer recommended". In histologically it is difficult to diagnosis of malignancy for pituitary adenoma, therefore invasion on pituitary adenoma was reflected on tumor size and/or associated with angiogenesis.

Response: We would like to thank you to point out that the major limitation of our study that we did not classify our specimens from patients using the most resent edition. In this revised manuscript, we explained in more details in the 2nd paragraph of Discussion section that the reason of lack complete results is the amounts of the specimens of part of our null cell adenoma (NCA) patients cannot perform additional assays of essential markers indicated by the 4th edition of the WHO classification unfortunately. We hope we can provide more information on NCA extremely using new collecting samples in the future. Furthermore, according to the suggestion of Reviewer #2, we altered the term “pituitary neuroendocrine tumors” to “pituitary adenoma”.

2. The authors concluded that "NCA progression occurs through ESM-1 associated angiogenesis". ESM-1 expression regulated by VEGF and FGF and VEGF is a key regulator of angiogenesis, which was the process of building new blood vessels. It is possibility ESM-1 expression was occurred by VEGF expression for angiogenesis of tumor.

Authors should describe more details of expression or amounts of another angiogenesis or invasive marker on sample collection. If angiogenesis marker, such as VEGF, were expression, it is possibility that ESM-1 expression was resulted for angiogenesis and VEGF expression. Please describe more details for availability of ESM-1 rather than VEGF and another markers.

Response: Because of the limited amounts of specimen, the expression of VEGF was not determined either in this study. As respect the assays we really conducted and the available results, we added the 3rd paragraph of Discussion to clarify that angiogenesis is just one of the possibilities of the mechanisms of ESM-1 involving invasiveness as follows: “The relationship among angiogenesis and ESM-1 expression in pituitary adenoma is still unclear. The expression of VEGF, the key regulator of angiogenesis was not evaluated in our study due to limited
available data and specimens. The expression of other important angiogenetic molecules, including FGF, stromal cell-derived factor, and the MVD was not detected. The possibility of ESM-1 doesn’t not involve in angiogenesis cannot be ruled out by our results. However, the invasiveness of tumor is certainly associated with the expression of ESM-1 via vascular structure.” We softened the descriptions of the relationship of ESM-1 and angiogenesis throughout the revised manuscript, too.

3. In addition, it is not clear for conclusion of this report. Did the authors suggest that "ESM-1 expression would become an invasiveness marker for pituitary NCA"? NCA was nonfunctional pituitary tumors and asymptomatic, therefor NCA was detected if size were large and progression. If the NCA tumor was detected at small size, did the ESM-1 expression in vascular endothelial tissues become the marker of enlargement of tumors? It was difficult to understand how ESM-1 expression in vascular endothelial tissues was became the marker of invasiveness. Please describe the definition of invasion on pituitary NCA and which marker ESM-1 expression was became, for example invasion, angiogenesis or enlargement of tumors.

Response: Rather than or in addition to an invasiveness marker, we believe that the role of ESM-1 may more complicated and need further studies to carry out. Both tumor size and invasiveness are related to the postoperative outcome, and our results indicated that significant positive associations were noted between ESM-1 expression in vascular endothelial tissues and tumor invasion and tumor size. However, only tumor size was associated with ESM-1 expression in adenoma tissues. With current data we still cannot certainly understand how ESM-1 play a role on both the changes of tumor size and invasiveness. Spontaneous shrinkage of the tumor remnants has been reported on nonfunctional pituitary adenoma, and MRI results was demonstrated that the direction and amount of blood supplies of tumor may be the predictors of spontaneous shrinkage. Thus we may hypothesize both vascular epithelial and adenoma ESM-1 involved in the alteration of tumor size, and it can be an easier assay compared than MRI. We incorporated a discussion of spontaneous shrinkage in the 6th paragraph of the Discussion section. However, we believe it’s too early to conclude the role of ESM-1. We want to thank you again for the above comment of angiogenesis, it inspires us to consider the possible role of ESM-1 more deeply.

Ronald J Benveniste (Reviewer 2): This is a moderate size study correlating ESM-1 expression in pituitary adenoma tumor and endothelial cells with clinically relevant imaging findings (extrasellar or suprasellar invasion, tumor size). The authors explain that the study included only null cell adenomas, in order to address conflicting results obtained in earlier studies that included many types of pituitary adenomas. The study is well designed and clearly reported, and the conclusions are definitely of interest to practicing clinicians.

Response: Thank you for your comment.
I recommend that the authors consider using the term "pituitary adenoma" rather than referring to pituitary neuroendocrine tumors, as this nomenclature is more commonly used in the US.

Response: As your suggestion, we changed the term “pituitary neuroendocrine tumors” to “pituitary adenoma” throughout the revised manuscript.