Author's response to reviews

Title: Thrombomodulin expression regulates tumorigenesis in bladder cancer

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

We are happy to learn that our manuscript, MS 1479901795122134, entitled “Thrombomodulin expression regulates tumorigenesis in bladder cancer” had been reviewed in BMC Cancer. We have included an itemized response to the reviewer’s comments, listed below.

Response to Reviewer #1:
1) Thanks for the reviewer’s suggestion, we added paragraphs in Introduction section to describe DNMT1 and NF-κB, “Several factors are implicated in regulation of TM expression including DNA methylation and nuclear factor-Kappa B (NF-κB) [21, 22]. We have previously reported that DNA methyltransferase1 (DNMT1) indicates more aggressive tumor growth and resistance to treatment in bladder cancer [23], and the activation of DNMT1 is enhanced by inflammatory cytokines [24]. Moreover, NF-κB is widely-recognized as a key regulator of the inflammatory responses, and plays an important role in various types of human cancers including bladder cancer [25-27]. Therefore, we proposed the inhibition of TM by NF-κB activation and DNMT1 might mediate in part the aggressive bladder tumor behavior.” (On page 5 line 9), and “the link between TM signaling, the activation of NF-κB and DNMT1 in bladder cancer was demonstrated.” (On page 5 line 18) in Introduction section

2) Thanks for the reviewer’s suggestion, we added Figure 7 to demonstrate our hypothesis, and the paragraph “By the data, we suggested that regulation of TM expression is critical in tumor aggressiveness and prognosis of bladder cancer. We outlined the main signaling pathways that are thought to link inflammation and TM signaling to the promotion of bladder cancer (Fig. 7).” (On page 18 line 17) in Discussion section

3) Thanks for the reviewer’s suggestion, we added the description in Introduction section “Although TM expression in tumor cells has been suggested to limit the invasive potential and proliferation of tumor cells in certain types of cancers, its role in bladder cancer remains to be elucidated.” and “Herein it is shown that decreased TM expression could predict the aggressive tumor growth and advanced clinical stage in bladder cancer. In addition, the link between TM signaling, the activation of NF-κB and DNMT1 in bladder cancer was demonstrated. The study highlight a potential role for TM as a molecular predictor and therapeutic target for bladder urothelial carcinoma.” We also added “Although TM has been suggested possessing prognostic value in some cancers [6, 10-12], the predictive role of TM in bladder cancer requires further investigation. Therefore, we evaluated tumor suppression role of TM in bladder cancer in the present study.” In Discussion section.

4) Thanks for the reviewer’s comment. We have rewritten some parts in Discussion section. Hope it could be better.
5) Thanks for the reviewer’s mention. We added one paragraph in Material & Methis to describe the detail about cell culture and transfection. “We maintained the bladder cancer cell lines in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. The TM silencing vector (the TM shRNA lentiviral transduction particles with puromycin resistance) and control vector (consisting of a non-effective scrambled shRNA cassette) were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA, USA). Stable TM-silenced cancer cells were generated by transfecting bladder cancer cells with the TM silencing vector and selected by culturing in medium containing puromycin for 4 weeks.” (On page 7 line 14)

6) Thanks for the reviewer’s mention. We added “(BA1=HT1197; BA2=HT1376; BA3=T24; BA4=J82)” in the Legend of Figure 1.

7) Thanks for the reviewer’s mention. We added “*” to indicate significance in the bar figures (Figure 1, 2, 4, 6).

8) As suggested by the reviewer, we changed the data of Western Blotting for cell lines in Figure 2a. Hope it could be better.

9) As suggested by the reviewer, we showed the IHC data in Figure 3c with high magnification. Hope it could be better.

Response to Reviewer #2:
Thanks for your comments.

We highly appreciate the reviewers’ precious comments that help to make this paper better quality. As above-mentioned, we have done the revisions in the paper according to their opinions. The changes that are in response to the Reviewers’ comments are shown in underlined text and for reorganization, grammar and spelling errors are shown in blue text in the revised manuscript.

Best regards

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