Peer Review File

Article information: http://dx.doi.org/10.21037/tau-20-1124

Review Comments

The paper titled "Identification of key genes and microRNA regulatory network in development and progression of urothelial bladder carcinoma" is interesting, which explored Identification of key genes and microRNA regulatory network in development and progression of urothelial bladder carcinoma However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: This study found that C8 + T cells, miR-450, miR-518s, transcription factors PAX3, KRAS and PTEN and urothelial bladder cancer are targetable markers. A lot of external experimental verification and clinical research are also needed.

Reply 1: Thanks for your kindly comments. This work is going to provide a comprehensive analysis for the ceRNA regulatory network in urothelial bladder carcinoma. We are validating these conclusions as well. But we thought this work was independent and would provide some clues for researchers. The external experiments and clinic research would be prepared later.

Comment 2: What kind of effect will activating the tumor immune microenvironment have on the prognosis of bladder cancer patients? Is there any good way to improve the prognosis of bladder cancer patients?

Reply 2: Regarding to the tumor immune microenvironment of bladder cancer, our analysis from the single sample Geneset enrichment analysis found that higher CD8+ T cells infiltrations were positive correlated with poor prognosis and tumor stages. The development and progression of tumor can somehow activated CD8+ T cells. From findings of both our analysis and previous researches, we can infer markers of activated CD8+ T cells like perforin and granzymes may serve as potential markers of bladder cancer outcome. Bladder cancer patients can benefit from adjustments of tumor immune microenvironment.

Comment 3: The study of combined detection of multiple tumor markers to diagnose bladder cancer is still in the early stage. How to enter the clinical use stage after continuous optimization?

Reply 3: Our study identified C8+ T cells, miR-450, miR-518s, transcription factor PAX3, KRAS and PTEN were highly correlated with urothelial bladder carcinoma's prognosis. In order to enter the clinical use stage, large-scale verification including external clinical samples are still in need. Furthermore, a more accurate, sensitive detection method still need to be optimized.

Comment 4: This study is entirely data analysis, and verification of the expression of key genes

should be added, which should be more convincing.

Reply 4: As we mentioned in reply 1, the verification work will be submitted as a new individual paper.

Comment 5: There have been many studies on urothelial bladder carcinoma. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply 5: Previous studies were mainly focused on single gene levels. We performed our analysis on functional related geneset level. By this way we can figure out changes during development and progression of urothelial bladder carcinoma from pathway and regulatory network level. The outcome of this analysis is better explainable than analysis on single gene level. By integrating data from genesets may also uncover new changes previously missing with single gene level analysis because this way alterations are enlarged by combining multiple genes.

Comment 6: It can increase the biological function and regulation mechanism of microRNA and key genes in bladder cancer.

Reply 6: Previous analysis based on single gene level may missing biological meaningful changes due to gene-level analysis are less obvious than geneset. Biological processes are realized through sets of genes functionally related. So by investigating expression alterations on genesets may help to uncover meaningful functional pathways and regulation mechanism at higher resolutions than single gene levels.

Comment 7: The discussion part is only a re-description of the results and a listing of the literature. It is recommended to add relevant possible mechanism to further enrich the content of the discussion.

Reply 7: Thanks for the remarks on discussion part. We added contents to the original discussion part with relevant literatures and a more detailed description of findings of our previous analysis.
Changes in the text: The 2nd and 3rd paragraphs in the discussion part.

Comment 8: Did the authors discover meaningful mutations in the process of data analysis?

Reply 8: Because this article focused on using single sample gene set enrichment analysis method to explain regulatory networks during development and progression of urothelial bladder carcinoma. This method now purely using expression data as input. As a result, we can only discover diverse in gene expression level but not in respect to mutations due to limitations of methodology.