Peer Review File

Article Information: https://dx.doi.org/10.21037/tcr-21-2700

Comment 1:

First, in the title "alterations" is not clear and vague, please indicate increase or decrease.

Reply 1:

Thank you very much. We have adjusted the title.

Changes in the text:

"Elevated expression of protein L-isoaspartate O-methyltransferase (PCMT1) in cervical cancer".

Comment 2:

Second, the abstract is not adequate. Please explain why PCTM1 deserved to be studied; lack of studies of PCTM1 in cervical lesions can not guarantee the necessity of the current research topic. Please use details figures and statistics to support the elevated level of PCMT1. In the conclusion please clearly indicate that prognostic biomarker or diagnostic biomarker. The conclusion in this part and elsewhere of the paper is problematic because elevated PCMT1 is not specific to cervical cancer.

Reply 2:

Thanks a lot. Though PCMT1 is not specific to cervical cancer, but it still can help to distinguish the cancerous and non-cancerous cervical tissue.We have rewritten the abstract.

Changes in the text:

Protein-l-isoaspartate O-methyltransferase-1 (PCMT1) is a protein carboxyl methyltransferase enzyme, which has been found to play roles in cancers. However, no clinical information about the correlation between cervical cancer and PCMT1 expression has been reported. We used immunohistochemistry (IHC) to characterize the protein level of PCMT1 in human cervical intraepithelial neoplasia and cervical cancer specimens. The mRNA expression profile of PCMT1 in cervical cancer was also analyzed by using Gene Expression Omnibus (GEO) databases. The prognostic value

of PCMT1 in patients with cervical cancer was evaluated by using the Kaplan-Meier plotter. Gene set enrichment analysis (GSEA) was conducted by using TCGA cervical cancer dataset. The protein level of PCMT1 was increased in cervical high grade squamous intraepithelial lesion (7.40 ± 0.42) and cervical cancer tissues (10.70 ± 0.54), compared to normal cervix (5.00 ± 0.86) and low-grade squamous intraepithelial lesion (LSIL) (6.22 ± 0.57) ($p < 0.05$). the IRS of PCMT1 was also higher in cervical cancer tissues than in paired adjacent non-cancerous cervical tissues (9.03 ± 0.52 vs. 6.32 ± 0.46) ($p < 0.05$). High expression of PCMT1 was associated with decreased overall survival of patients with cervical cancer ($p = 0.0022$). GSEA demonstrated that cervical cancer patients with high expression of PCMT1 were enriched in the various cancer-related signaling pathways. These results suggest that PCMT1 might be a diagnostic and prognostic biomarker for cervical cancer, and further validation studies should be performed.

Comment 3:

Third, the introduction part is written in a confused way: in first paragraph the authors emphasize therapeutic targets but in the third paragraph they emphasized prognostic factor. Please have a brief overview of known prognostic biomarkers of cervical cancer and comments on the limitations of previously known biomarkers and the potential strengths of PCMT1, to indicate the necessity of the current research topic. Please explain why there is a need for a focus of regulatory signaling pathways of PCMT1 in cervical cancer.

Reply 3:

Thanks a lot. According to the suggestion, we have modified the first and third paragraphs of the “introduction” section.

Changes in the text:

Among women, cervical cancer is the fourth most frequently diagnosed tumor worldwide, with an estimated 0.57 million cases and 0.31 million deaths according to the latest global cancer statistics (1). At present, the therapeutic strategies for cervical cancer include surgery, chemotherapy, radiotherapy and combined therapy. Although

great improvements have been attained in the treatment of cervical cancer, the survival rate of patients with advanced disease remains poor (2). At present, a variety of molecular biology, genetics, targeted antitumor drugs and diagnostic, prognostic and predictive biomarkers are being sought. Ki-67, cyclin D1, p53, p63 and p16INK4a are widely used in the clinical work but insufficient in r differential diagnosis and prognosis estimation (3). Finding reliable genetic, molecular and immunohistochemical markers for early diagnosis of cervical precancerous/cancerous lesions and neoplastic processes, as well as prognosis estimation remains an important task.

In current study, we focus on the alterations in the expression of PCMT1 in cervical cancer. Immunohistochemistry and public databases analysis were used to show the expression pattern of PCTM1 in different pathological types of cervical tissues. The prognostic value in cervical cancer were also explored. To further understand the role of PCMT1 in cervical carcinogenesis, the potential related regulatory signaling pathways were explored.

Comment 4:

Fourth, in the survival analysis of methodology part, please explain the subjects inclusion, outcomes, and follow up procedures of the cohort used for the survival analysis.

Reply 4:

The Kaplan-Meier plotter (http://kmplot.com/analysis/) database was used to perform survival analyses of cervical cancer patients. The Kaplan Meier plotter is capable to assess the effect of 54k genes on survival in 21 cancer types. The system includes gene chip and RNA-seq data-sources for the databases include GEO, EGA, and TCGA. Primary purpose of the tool is a meta-analysis based discovery and validation of survival biomarkers. We did not use the cohort of patients in our hospital for the survival analysis. Thus, we don't have the details of subjects inclusion, outcomes, and follow up procedures.

Comment 5:

Finally, in the statistics, please indicate the groups to be compared by using t test and $P<0.05$ is two-sided.

Reply 5: Thank you very much, we have adjusted the text.

Changes in the text:

The Student's t test was used to compare the gene expression between different groups. Kaplan-Meier analysis was used to evaluate the survival rate of cervical cancer patients. Statistical analyses were performed in GraphPad Prism (Version 8.0, GraphPad Software, La Jolla, CA, USA). A p-value < 0.05 (two-sided) indicated a statistically significant difference