Peer Review File

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Reviewer A

Comment 1:

First, the title needs to indicate the development and validation of the prognosis prediction model and the dataset used for the development of the model.

Reply 1:

Thanks for the suggestion, we have changed the title as follow: Development and Validation of a Prognostic Prediction Model for Cervical Cancer Patients Treated with Radical Radiotherapy: A study based on TCGA Database.

Comment 2:

Second, the abstract is inadequate. The background did not describe the limitations of prior studies of the prognosis prediction models of CESC. The methods need to describe the generation of training and validation samples, prognosis outcomes and how the predictive validity was analyzed. The results need to briefly describe the clinical sample and the prognosis of the clinical sample, as well as the predictive accuracy parameters in both the training and validation samples. The current conclusion should be tone down due to the limited data on external validity.

Reply 2:

Thanks for the suggestion, we have changed the abstract as follow: **Background:** Radiotherapy or concurrent chemoradiotherapy is the standard treatment for patients with locally advanced or inoperable cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC). However, treatment failure for CESC patients treated with radical radiotherapy still occurs due to local recurrence and distant metastasis. The previous prediction models were focused on all CESC patients, neglecting the prognostic differences under different treatment modalities. Therefore, there is a pressing demand to explore novel biomarkers for the prognosis and sensitivity of radiotherapy in CESC patients treated with radical radiotherapy. As a single biomarker has limited effect in stratifying these patients, our objective was to identify radioresponse-related mRNAs to ameliorate forecast of the prognosis for CESC patients treated with radical radiotherapy. **Methods:**

Sample data on CESC patients treated with radical radiotherapy were obtained from the Cancer Genome Atlas (TCGA) database. We randomly separated these patients into a training and test cohorts using a 1:1 ratio. Differential expression analysis was carried out to identify radioresponse-related mRNA sets that were significantly dysregulated between complete response (CR) and radiographic progressive disease (RPD) groups, and univariate cox regression analyses, least absolute shrinkage and selection operator (LASSO) method and multivariate cox regression were performed to identify the radioresponse-related signature in the training cohort. we adopted survival analysis to measure the predictive value of the radioresponse-related signature both in the test and entire cohorts. Moreover, we developed a novel nomogram to predict the overall survival of CESC patients treated with radical radiotherapy. In addition, immune infiltration analysis and Gene Set Enrichment Analysis (GSEA) were conducted to preliminarily explore possible mechanisms. **Results:** This study included a total of 92 CESC patients subjected to radical radiotherapy. We developed and verified a risk score model based on radioresponse-related mRNA (PTX3, LRRC66, CERS4, SLC4A11, and FMOD). The radioresponse-related mRNA signature and FIGO stage were served as independent prognostic factors for CESC patients treated with radical radiotherapy. Moreover, a nomogram integrating radioresponse-related mRNA signature with FIGO stage was established to perform better for predicting 1-, 3-, and 5-year survival rates. Mechanically, the low-risk group under the risk score of this model had a better survival status, and the distribution of CD4 T cells was potentially involved in the regulation of radiotherapy response in CESC, leading to a better survival outcome in the low-risk group. **Conclusions:** This study presents a new radioresponse-related mRNA signature that shows promising clinical efficacy in predicting the prognosis of CESC patients treated with radical radiotherapy.

Comment 3:

Third, in the introduction, please review the known prognostic biomarkers and available prognosis prediction models for CESC and have detailed comments for their limitations and knowledge gaps in particular their predictive accuracy.

Reply 3:

Thanks for the suggestion, we have changed the introduction as follow: Cervical cancers are one of the most common malignant tumors among women. It is estimated that in the United States in 2023, 1,958,310 people were diagnosed with cancer, among them, with 13,960 diagnosed with

cervical cancer (CC) (1). Among cervical cancers, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) comprise 10-15% of all female cancer-related mortalities and are the second most fatal malignancy in women (2). In most instances, patients have already progressed into locally advanced stages when the diseases were first definitely diagnosed. Radiotherapy or concurrent chemoradiotherapy is the standard treatment for patients with locally advanced or inoperable disease (3). Local recurrence and distant metastasis are still the main causes of treatment failure for CESC patients treated with radical radiotherapy (3,4). Thus, there is an urgent need to investigate novel biomarkers for prognosis and sensitivity of radiotherapy in these patients. However, the previous prediction models were focused on all CESC patients, neglecting the prognostic differences under different treatment modalities, leading to poor predictive accuracy (5-7). Consequently, a multivariate tool is necessary for predicting the prognosis and sensitivity of radiation to guide suitable treatment for CESC patients undergoing radical radiotherapy.

We also add some comments in the discussion part as follow: Cervical cancer is a prevalent malignancy among women, with cervical squamous cell carcinoma (CESC) comprising 10-15% of all female cancer-related mortalities and being the second most fatal malignancy in women (1,2). The primary treatment options for cervical cancer include surgery, radiation therapy, and chemotherapy. For patients with locally advanced or inoperable disease, radiotherapy or concurrent chemoradiotherapy is the standard radical treatment (3,16). Despite this, the prognosis for CESC patients treated with radical radiotherapy remains poor, with local recurrence and distant metastasis being the primary causes of treatment failure (3,4,17). Hence, there is a pressing need for the identification of novel biomarkers for the prediction of prognosis and sensitivity to radiotherapy in these patients. mRNAs, which encode proteins involved in numerous cellular processes, hold a pivotal position in the progression, relapse, and spreading of cancerous cells. Given their central role in cellular metabolic processes, the selection of appropriate mRNA biomarkers is of significant importance (18-20). Single biomarker may not fully capture the heterogeneity and complexity of the tumor, and thus a multi-parameter approach is necessary to achieve more accurate predictions. However, the previous prediction models were focused on all CESC patients, neglecting the prognostic differences under different treatment modalities, leading to poor predictive accuracy. In addition, considering other studies that take into account additional

functional backgrounds such as immune-related factors, DNA damage repair-related factors, and necroptosis-related factors when screening candidate genes, these selected genes may not possess the most optimal prognostic predictive effect (5-7). In this study, we have constructed a multivariate tool to predict the prognosis and sensitivity of radiation therapy and to guide appropriate treatment for CESC patients treated with radical radiotherapy.

Comment 4:

Fourth, in the methodology of the main text, please briefly describe the research procedures of this study, as well as the details of the clinical sample in the dataset. In statistics, please analyze the statistical power of the small sample used to develop and validate the prediction model. I do not think this sample is adequate. Please also describe the threshold AUC values for a good prediction model and the calculation of the 95%CIs of the AUC values since the sample is very small. Please also ensure $P<0.05$ is two-sided.

Reply 4:

Comment4_1(In the methodology of the main text, please briefly describe the research procedures of this study, as well as the details of the clinical sample in the dataset).

Reply4_1:

Thanks for the suggestion, we have changed the methods part as follow:

2.1 Datasets and Processing

The mRNA seq expression profiles of TCGA-CESC and their associated clinical data were gathered from the TCGA project (https:/portal.gdc.cancer.gov/). The gene expression screening condition were “Project: TCGA-CESC”, “Data Category: Transcriptome profiling”, “Data Type: Gene Expression Quantification”, “Experimental Strategy: RNA-Seq”, “Workflow Type: STAR - Counts”. The clinical information screening condition were “Project: TCGA-CESC”, “Data Category: Clinical”, and “Data Type: Clinical Supplement”. The transcriptional profiles and clinical data of CESC are publicly accessible and offered on an open-access basis. Hence, authorization from a local ethics committee was deemed unnecessary. The gene annotation information for all genes was sourced from the human GENCODE project (https://www.gencodegenes.org/).

This study included a total of 92 CESC patients subjected to radical radiotherapy. The inclusion criteria for all cases were as follows: 1) all patients were diagnosed with CESC histologically; 2)

radiotherapy was performed as the radical treatment for CESC patients; 3) expression profiles were available; and 4) the patient's overall survival time was more than 30 days. It should be mentioned that only 57 individuals had the curative effect evaluation. Thirty-six patients were evaluated as complete response (CR), seven patients were evaluated as partial response (PR), two patients were classified as stable disease (SD), and 12 patients were classified as radiographic progressive disease (RPD). The details of the clinical sample in the dataset were shown in Table S1.

The research procedures of this study were as follow: Firstly, we obtained radioresponse-related mRNAs in CESC patients treated with radical radiotherapy. Secondly, we screened five radioresponse-related mRNAs in the training set and established a robust 5-gene prognostic signature. Then, we employed both the test cohort and the entire cohort to validate the prognosis outcomes of our signature. Additionally, we formulated a nomogram model by integrating the signature with other relevant clinical factors. Finally, immune infiltration analysis and Gene Set Enrichment Analysis (GSEA) were conducted to preliminarily explore possible mechanisms.

Comment4_2(In statistics, please analyze the statistical power of the small sample used to develop and validate the prediction model. I do not think this sample is adequate).

Reply4_2:

Thanks for the suggestion, we completely understand your concerns about the sample size and agree that it is an important consideration. In previously published articles on prognosis models for cervical cancer, most of them have used the GSE44001 dataset for external validation, which consists of patients with early-stage cervical cancer who underwent surgery and does not match our study population. Due to the lack of transcriptomic and clinical information databases specifically for cervical cancer patients treated with radiotherapy, we were indeed limited to a relatively small sample size. Despite this limitation, we made efforts to obtain meaningful results through rigorous methodological design and reliable data analysis.

Comment4_3(Please also describe the threshold AUC values for a good prediction model and the calculation of the 95%CIs of the AUC values since the sample is very small.).

Reply4_3: Thanks for the suggestion. However, there is no universally defined threshold for determining what constitutes a good AUC score. It is evident that the higher the AUC score, the better the model's ability to classify observations into their respective classes. Moreover, it is

generally understood that larger sample sizes contribute to increased accuracy in the model's performance. In addition, we added the calculation of the 95CIs of the AUC values in the results (nsROC package of R).

Comment4_4(Please also ensure P<0.05 is two-sided).

Reply4_4:

Thanks for the suggestion. We would like to clarify that all our P-value analyses are indeed two-sided. We have ensured that the statistical tests performed in our study account for both directions of the hypothesis, and the reported P-values reflect this consideration.

Comment 5: Finally, please cite some related papers:

1. Guaraldi L, Pastina P, Tini P, Crociani M, Marsili S, Nardone V. Locally advanced cervical cancer treated with chemo-radiotherapy: a case report of a particular recurrence. Gynecol Pelvic Med 2021;4:30..
2. Liu YY, Zhu WH, Li W, Hao X, Zheng W, Shen Q. Identifying the risk factors and developing a predictive model for postoperative pelvic floor dysfunction in cervical cancer patients. Transl Cancer Res 2023;12(5):1307-1314. doi: 10.21037/tcr-23-385.
3. Jiang Z, Zhang G, Sun T, Zhang G, Zhang X, Kong X, Yin Y. Advantages of IMRT optimization with MCO compared to IMRT optimization without MCO in reducing small bowel high dose index for cervical cancer patients—individualized treatment options. Transl Cancer Res 2023;12(12):3255-3265. doi: 10.21037/tcr-22-2792.

Reply 5: Thanks for the suggestion, we have cited these related papers in our manuscript.

Reviewer B

1. Please provide the full names of FMOD and FIGO in the abstract.

   Reply：Thanks for the suggestions, we have revised our manuscript.

2. The citation of Figure 9F and 9I is missing in the text. Please check and revise.

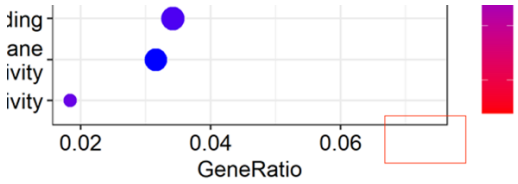
   Reply：Thanks for the suggestions, we have revised our manuscript.

3. Table S1: please check the P value = 1. It is suggested to revise it to "> 0.99".

P value	Test
0.662	Ranksum test
1	Chi-square test

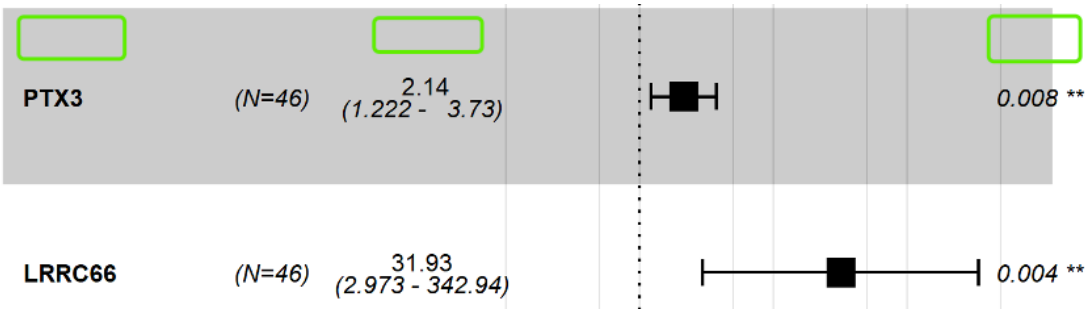
Reply：Thanks for the suggestions, we have revised our manuscript.

4. Figure 3b: Please complete the scale bar.



Reply： Thanks for the suggestions, we have revised it.

5. Figure 4C: It is suggested to complete the heads.

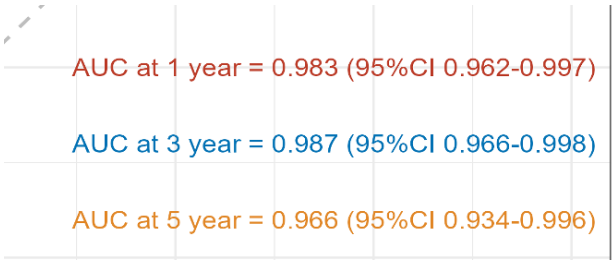


Reply：Thanks for the suggestions, we have revised it.

6. Figure 4c: Please indicate the meanings of * and **.

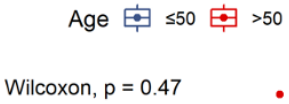
Reply：Thanks for the suggestions, we have revised it.

7. Figure 4f, 5c, 5f, 5h: It is suggested to revise 3 year; 5 year to 3-year; 5-year.



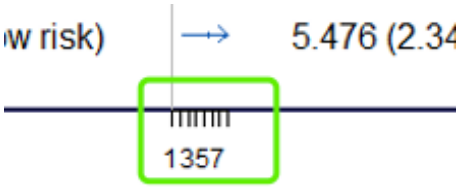
Reply：Thanks for the suggestions, we have revised it.

8. Figure 6b, 7a, 7b: Please check if any unit should be added to the age.



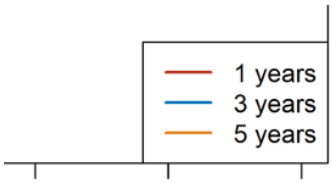
Reply：Thanks for the suggestions, we have revised it.

9. Figure 7A-B: the scale bars are too close. Please modify.



Reply：Thanks for the suggestions, we have revised it.

10. Figure 7d: Please revise 1 years to 1 year.



Reply：Thanks for the suggestions, we have revised it.

11. Figure 9 legend: the cell names don't match with the figure.

Reply：Thanks for the suggestions, we have revised it.

601 Correlation between signature and Macrophages M2 (b), T cells CD4 memory resting
602 (c), T cells CD4 memory activated (d), and T cells gamma delta (e). Kaplan–Meier
603 curves for Macrophages M2 (f), T cells CD4 memory resting (g), T cells CD4 memory
604 activated (h), and T cells gamma delta (i) in CESC patients underwent radical
605 radiotherapy.

12. Figure 9a: Please indicate the meaning of *, ** and *** in the legend.

Reply：Thanks for the suggestions, we have revised our manuscript.