

## Peer Review File

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### Reviewer A

1. In Figure 1G, the authors suggested that HPV may activate HACE1 expression to exert oncogenic effects. However, the data provided is insufficient to support this conclusion.

**Response:** We recognize the imprecision of this statement. Indeed, as the reviewer stated, this conclusion cannot be reached based on the correlation results, so we removed this sentence from the results.

2. The conditions of the IHC staining for HACE1 should be described, including the titer, temperature, and staining time. Additionally, it is important to indicate whether these patients harbored HPV-positive cervical cancer, as this could provide valuable context for understanding the expression patterns of HACE1.

**Response:** Thank you for your correction! We have added a detailed description in the methods section (**Page 5, line 180-187**) and indicated that these 6 patients were HPV-positive.

3. The figure legend of Figure 1G should be corrected for accuracy.

**Response:** Thanks for pointing out the error, we have changed the labeling of the legend (**page11, line460-461**).

4. In Figure 2, the method for selecting the cut-off value of HACE1 should be described.

**Response:** We have supplemented the method for selecting the HACE1 cutoff value in the Methods section and added the number of patients in the high expression group and low expression group in Figure 2 (**page4, line160-162**).

5. It should be clarified which survival time data (e.g., OS, RFS) was used for the Kaplan-Meier survival curve and multivariate Cox analysis.

**Response:** We mainly use OS as a prognostic indicator for Kaplan-Meier survival curves and multivariate Cox analysis, this description has been added in the Methods section (**page4, line162-164, page6, line219-220**).

6. In Figure 3A, it should be clarified whether HACE1 was selected as a continuous variable or categorized as high versus low.

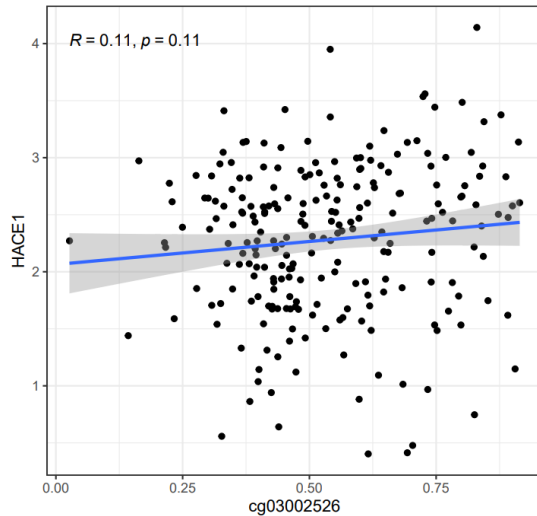
**Response:** Thanks for pointing it out! HACE1 was included as a continuous variable in the multifactor COX assessment, and a detailed description has been added in the results section. (**page12, line473-474**).

7. Additionally, the method for selecting the cut-off value of HACE1 should be described.

**Response:** Since multivariate COX analysis is based on continuous variables, the cut-off value is limited to the survival analysis in Figure 2. The relevant modifications have been replied to question 4.

8. The correlation between the beta value of cg03002526 and HACE1 expression should be presented in the results.

**Response:** Thanks to the reviewer's suggestion, we conclude that the methylation level of cg03002526 in HACE1 is significantly down-regulated in HPV+ patients, suggesting that HPV infection affects the methylation of cg03002526. However, through correlation analysis, we did not find a statistically significant correlation between the expression of HACE1 and cg03002526. This indicates that the methylation level of cg03002526 in HACE1 is mainly affected by HPV infection, but this effect is only between HPV+ and HPV- patients, and there is no linear expression relationship based on the population. For this negative result, we have adjusted the description of the results to make the conclusion more accurate. (**page7, line274-275**).



## Reviewer B

1. The main text should be structured as **Introduction**, Methods, Results, Discussion and Conclusions. Please modify your article to it.

Reply:

We have changed "Background" to "Introduction". (Page3 line111)

2. Please double check and revise the full name of “TCGA-CESC” in the abstract and the main text.

**Methods:** From The Cancer Genome Atlas (TCGA-CESC) and Gene Expression Omnibus (GEO, GSE6791) datasets, we obtained RNA-Seq profiles and associated clinical

**I Data Sources and Selection:**↵

Cervical cancer dataset (TCGA-CESC) from the TCGA database (<https://portal.gdc.cancer.gov/>) encompassed 306 RNA-Seq profiles and associated

Reply:

We have completed the modifications in the corresponding locations. (Page2 line60,61, Page4 line153,154)

3. Please unify the Hospital name in the whole manuscript.

revised in 2013). The study was approved by Ethics Committee of The People's Hospital of Chongqing Liangjiang New Area (No. 2023-19) and individual consent for this retrospective analysis was waived.↵

Six cervical cancer HPV-positive samples and six normal cervical tissue samples were obtained from the Pathology Department of Liangjiang New Area People's Hospital in Chongqing. Samples were subjected to deparaffinization, antigen retrieval, endogenous

Reply:

We have completed the modifications in the corresponding locations. (Page5 line186,187)

4. The main text should be structured as **Introduction**, Methods, Results, Discussion and Conclusions. Please modify your article to it.

Reply:

We have responded in question 2 (this question is the same as question 2.

5. Please revise all p to “P” in the whole manuscript.

Reply:

We have completed the modifications in the corresponding locations.

Page4 line176

Page5 line203,209,213,218

Page6 line227,229,230,231,241,280

Page7 line278

Page11 line452,455

Page12 line458,461,461,462,479

Page13 line501

6. Figures

- Figures should be cited **consecutively** in the text and **numbered in the order** in which they are discussed. For example, Figure 1A, Figure 1B, Figure 1C... Please check through and revise.

212 We observed significant upregulation of HACE1 in tumor samples (Figure 1A,  $p < 0.0001$ ).  
213 Immunohistochemical staining of cervical cancer and normal cervical tissues revealed  
214 stronger HACE1 staining in tumor tissues (Figure 1H). Subsequently, based on previous  
215 reports on HPV subtyping in the TCGA-CESC, we found upregulated HACE1 expression  
216 in HPV-infected patients (Figure 1B,  $p < 0.05$ ), but no statistically significant differences

Reply: We have modified the layout of Figure 1 to match the writing order in the manuscript, and we have also made appropriate modifications to the description of the manuscript. (Page5 line210-221)

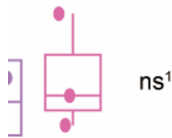
- **All abbreviations** in figures and legends should be explained. HACE1, HPV, FIGO, neg., and pos. in Figure 1 for example. Please check **all abbreviations** and provide the full names in the corresponding legends.

Reply: We have added the full name of the abbreviation in the relevant legend.

Page12 line462-465, 473-475, 485-487,494-496

Page13 line503-505

- Figure 1C: Please indicate the meaning of <sup>1</sup>.

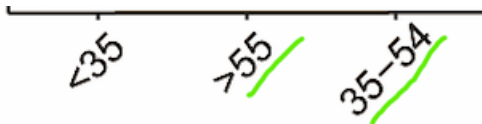


Reply: We added a description about "<sup>1</sup>" in the legend (analysis of variance with HPV16 type as control) (Page12 line457-458)

- Figure 1F, 2D, 3A: Please add a **unit** for age.

Reply: We have added unit for age. (Figure 1E, 2D)

- Figure 1F, 2D, 3A: should the age be “35-55” or “>54”? Please check.

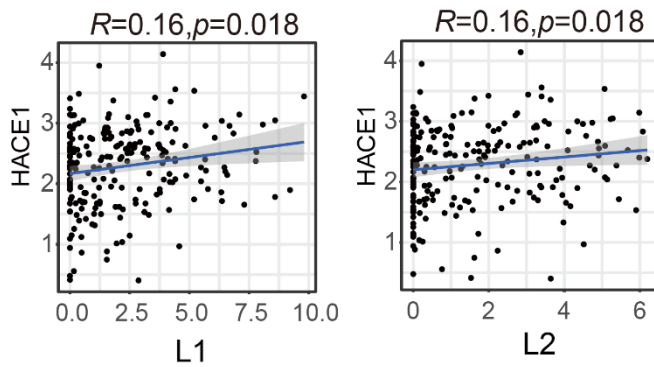


Reply: This was our mistake, we have changed "35-54" to "35-55" in the Figures. (Figure 1E, 2D)

- Figure 1G: The data of the following two graphs are **the same**. Please check if they are correct.



Reply: Thank you for pointing it out. We have re-confirmed that there is no problem with the graph and statistics (the specific reason is that L1 and L2, as the capsid proteins of the HPV virus, are highly co-expressed, so the very close statistical results are consistent with common sense.) In addition, we carefully checked the pictures and found that they were not the same picture (the points in the pictures were not at the same position). (Figure 1H)



- Figure 1H: Please indicate the **staining methods** and the **magnification**.

Reply: We have made corresponding modifications in Figure 1B and added a description of the magnification factor in the legend.

- Figure 2A: The numbers can't be seen clearly. Please modify.

h	206	161	100	61	37
n	87	68	42	31	25

Reply: The corresponding picture in Figure 2A has been modified for ease of reading.

HACE1	High	206	161	100	61	37	26
	Low	87	68	42	31	25	18
		0	1	2	3	4	5

- Figure 3A, 4A: Please revise all pvalue to **P value**, Hazard ratio to **Hazard ratio (95% CI)**.

**pvalue**

**Hazard ratio**

Reply: We have made the modifications in the Figure 3A.

- Figure 3D: Please check if the description of Y-axis should be 1 Year; 3 Years and 5 Years.

Fraction Surviving 1 Days  
0.0 0.2 0.4 0.6 0.8 1.0

Fraction Surviving 3 Day  
0.0 0.2 0.4 0.6 0.8 1.0

Fraction Surviving 5 Day  
0.0 0.2 0.4 0.6 0.8 1.0

Reply: We have fixed this error in the Figure 3A

7. Please check if more references should be cited since you mentioned *studies*.

- *Studies* have indicated HACE1's interaction with Rac1 and its ubiquitination at its lysine residues, modulating processes such as cell motility, protein translation, and cell growth<sup>8</sup>.
- However, some *studies* suggest that HACE1 is not a tumor suppressor in NK cell malignancies<sup>19</sup>.

Reply: For the first sentence mentioned, we have added relevant references. For the second sentence, we changed the relevant writing. (Page3, line129,Page10, line391-392, Page7, line294)