Peer Review File

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Reviewer A

This study explored methylation-driven genes in cervical squamous cell carcinoma that is an emerging and relevant topic in gynecological cancer.
The authors found 144 differentially expressed methylation-drive genes in CESC and ITGA5, HHEX and S1PR4 genes were screened out by Cox regression analyses and used to construct a prognostic risk model.
The group of research concluded that reliable bioinformatic basis of methylation-driven genes and identified ITGA5, HHEX and S1PR4 as key genes which might serve as biomarkers or prognosis risk assessment model and therapeutic targets in CESC.
This study is well written, is clear and bibliography are adequate.
Materials and methods sections are precise and complete concerning the contents and this is the strongest aspect of the manuscript.
The English language is correct and pertinent for scientific manuscript.
For these reasons, I think that this manuscript should be accepted for publication.

Answer: Thanks for your precious comment.

Reviewer B

In this article, authors report the employment of a great variety of bioinformatic tools to analyze available datasets of previous studies on cervical cancer tissue collections. Although the article undoubtfully contains original knowledge and significant information, it has several limitations, mostly related to structure, content and language that should be improved before it reaches publication quality.

Answer：Thanks for your kind suggestions! We have improved the quality of content and language of our article. The language editing certificate has supplemented.

Language is generally poor and needs significant revision. Only some spotted mistakes:
L8 Abnormal methylation of genes is
L19 helped
L24 methylation
L 66 cancers
L 80 evidence
L 81 genes
L 82 genes
L 90-92 rephrase
L 347-349 rephrase

Answer：Thanks for your comments! We are very sorry for the incorrect writing, and we have revised them. The spotted mistakes were revised:

L35

L41-44

L47

L91-94

L98-100

The other mistakes were also corrected and labeled in yellow color. Please check them in the text! Thank you again.

The abstract has to be rewritten in a way to better represent findings and conclusions. The exclusive bioinformatic approach in the data construction of the study has to be clearly stated. I suggest also in the title. L44 therapeutic targets? Is it justified by data?

Answer: Thanks for your valuable comments! The abstract has been rewritten. In the present study, there was lack of experimental evidence to prove the three key genes (ITGA5, HHEX and S1PR4) could serve as therapeutic targets. However, in view of the prognostic value of the key genes, we speculate that these genes might be involved in the progression of cancer and serve as potential therapeutic targets. We have revised it, and thank you again.

Changes in text: methods, results and conclusions part of the abstract.

Introduction

Lengthy and somewhat general. Please focus in studies adopting similar approaches and their input to the field.

Answer: Thanks for your comments, we have revised it. Please recheck it.

Changes in text: L80-83, L88-94 and L99-101 in the introduction part.

M&M

106-111. Please provide further information for the datasets used. How can the reader identify them? Are they the only relevant datasets available in TCGA? If not, how sere they chosen, based on which criteria?

Please prove a table with patients' characteristics, clinipathological and demographic data. These data were acquired from cervical smears?

Answer: Thanks for your kind advices. We have provided further information for the data used in our study. We downloaded the expression profiles of mRNAs (level 3) in 309 cases (306 tumor tissues and 3 normal tissues), the methylation data level 3) in 312 cases (309 tumor tissues and 3 normal tissues) from TCGA database (http://cancergenome.nih.gov/). The sequenced data were obtained from Illumina HiSeq RNASeq and Illumina Human Methylation 450 platform. In addition, we provided a table with patients' characteristics downloaded from TCGA database in **Supporting table 1.** Above data were downloaded on oct 2018. These data in TCGA database were provided by National Cancer Institute (NCI) of the U.S.A, and samples were acquired from surgical samples.

Changes in text: L106-113.

Discussion

The Discussion is generally lengthy. Are there any similar signatures in CESC and/or other cancer types? Have they been introduced to clinical diagnostics?

the 349-355 There is plenty of literature for the predictive value of methylation specifically in CESC and it would be more appropriate to reference.

Answer: Thanks for your attention on our study. Previous researches have defined and demonstrated numbers of methylation-driven genes played crucial roles in progression of CESC and other cancers. We have supplemented the researches about the similar signature in the discussion part. The biomarker identified and the risk signatures in these studied provided novel idea for clinical diagnostics, but need more evidences to prove that.

Changes in text: in L313-316.

358 Rephrase. Also, it is not justified from findings that effort was put in identifying therapeutic targets. Gene up- or down-regulation might be the result of the carcinogenetic sequel of events. To prove therapeutic opportunity functional data should indicate that reversing events would reverse phenotype.

Answer: Thanks for your precious suggestions, we have rephrased the sentences. For the absence in the technology, time and funding, the function assays of these candidate genes have not been performed in the present study. However, we will continue to investigate these significant candidates in CESC to provide new ideas for clinical application. Thanks for your valuable suggestion sincerely.

Changes in text: L316-317

375-376 Rephrase. Explain better. 361-376 the paragraph does not smoothly lead to conclusions.

Answer: Thanks for your precious suggestions, we have rephrased the sentences. Please check it. Thank you again.

Change in text: L327-329.

Reviewer C

1. Whether the results of KEGG are correlated with those of GSEA.

Answer： Thanks for your attention on our article. The KEGG analysis devoted to investigate the underlying regulation mechanism of a group of candidate genes, while the GSEA uncovered the enriched pathways of one target gene.

2. “ The stratification analysis was performed based on age, grade, histological type and stage.” But I don't understand why the age = 50 was regarded as a value of cut off. In addition, the information in Figure 8D did not match the legend.

Answer： Thanks for your precious advices! We identified age = 50 as the cut off according to the globally average age of cervical cancer was about 50 years-old based on the worldwide analysis in 2018.(Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, Bray F. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health. 2019 Dec 4. pii: S2214-109X(19)30482-6. doi: 10.1016/S2214-109X(19)30482-6.). In addition, we have revised the legends of Figure 8D. We are very sorry for our mistaking writing. Thanks for your valuable suggestion sincerely.

Changes in text: L518-519

3. This bioinformatics study lacked independent data validation.

Answer：Thanks for your valuable suggestions! According to the absence of corresponding prognostic clinical information in GEO database, independent data validation could not be provided in the present study. we have supplemented this defect in the discussion part, further validation in other independent data and experimental evidences.

Changes in text: L370-L375.