nature portfolio

Peer Review File

Acetyl-CoA carboxylase 1 knockdown promotes esophageal squamous cell carcinoma metastasis via the CXCL8–NET axis

Corresponding Author: Professor Baosheng Zhao

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In this study, Tang et al. focused on the function of ACC1 in ESCC metastasis progression of ESCC, in which they identified the key role of ACC1-CXCL8-NETs axis. Generally, the current results provide good support for the conclusion in this study. However, some issues need to be addressed seriously.

1.In the section of “MATERIALS AND METHODSA”, authors stated that IHC was performed based on the tissue microarray (TMA) containing 97 cases of ESCC, however, no relevant results, especially the protein levels of ACC1, were displayed in the manuscript.
2.In Figure 1A, authors analyzed ACC1 mRNA levels in the group with good or poor prognosis, which missed the detailed standards about the definition about different prognosis. In Figure 1C, the correlation between ACC1 protein levels and clinical prognoses is more worthy of evaluation.
3.In Page 13 Line 281, the description about “an independent predictor” should result from the Multivariate Cox Regression analysis.
4.The scale bar in the images from transwell assays should be indicated clearly.
5.The spelling mistake about “Metastesis” in Figure 7E should be corrected.
6.The grammar in this manuscript needs to be carefully proofread.

Reviewer #2

(Remarks to the Author)
Dear Author,
I sincerely appreciate the opportunity to review this manuscript. In the present study, the authors focus on the role of Acetyl-CoA carboxylase 1 (ACC1) in esophageal squamous cell carcinoma (ESCC). When ACC1 knockdown in ESCC cells, it promotes epithelial-mesenchymal transition (EMT) and enhances cell migration and invasion by activating the PI3K/AKT and MEK/ERK pathways through CXCL8 up-regulation. I think this research is well written and the experimental design is reasonable. However, there are some faults that the authors need to amend and explain. For example, in Page 9, "Western blot" section, after PVDF membranes transferred, the PVDF membranes need be blocked with fat-free milk or BSA. Furthermore, the authors claim that "Total protein of 20 μg for each sample was separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) ..." in Page 9, but the images of Western blot look like something else. For example, in Fig. 4, the "beta-actin" protein bands in KYSE 150 and 450 don't like the images of Fig.S 2 A & B and other Figures in "Original western blots". Therefore, this manuscript should be carefully revised to be suitable for publication.

Reviewer #3

(Remarks to the Author)
The paper identifies a new mechanism by which ACC1 knockdown promotes esophageal squamous cell carcinoma (ESCC) metastasis through the CXCL8-NETs axis. It demonstrates that ACC1 expression is associated with poor prognosis in ESCC patients and that ACC1 knockdown enhances ESCC cell migration and invasion in vitro. Furthermore, it verifies the role of the ACC1-CXCL8-NETs axis in ESCC metastasis using xenograft tumor models and lung metastasis models.

The topic is interesting and the data analysis is convincing.

The Minors Concerns:
1. It would be more compelling to use immunohistochemical analysis for testing the prognostic value of ACC1 in ESCC patients.
2. While the study shows that ACC1 knockdown promotes ESCC metastasis via the CXCL8-NETs axis, the specific molecular mechanisms underlying ACC1's regulation of CXCL8 require further exploration. For example, are there other transcription factors or signaling pathways involved in addition to c-Fos?
3. The author should explore the therapeutic potential of targeting the ACC1-CXCL8-NETs axis by evaluating the effects of CXCL8 inhibitors or NETs inhibitors on ESCC metastasis in preclinical models.
4. The author should analyze the correlation between the expression of relevant molecules and clinical outcomes in a larger cohort of ESCC patients to validate the clinical efficacy of these therapeutic targets.

Version 1:

Reviewer comments:

Reviewer #1

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The issues have been appropriately resolved in the revised manuscript. However, the explanation about scale bars in the images from transwell assays was still not found in the figure legends of Figs.2-6.

Reviewer #2

(Remarks to the Author)
Dear Authors,
In the present study, the authors focus on the role of Acetyl-CoA carboxylase 1 (ACC1) in esophageal squamous cell carcinoma (ESCC). When ACC1 knockdown in ESCC cells, it promotes epithelial-mesenchymal transition (EMT) and enhances cell migration and invasion by activating the PI3K/AKT and MEK/ERK pathways through CXCL8 up-regulation. I think this research is well written and the experimental design is reasonable. The authors also revise the manuscript according to the reviewers' comments. Therefore, I suggest that this manuscript could be published in this journal.

Chief Editor

Communications Biology

July 23, 2025

Dear Editor,

On behalf of my co-authors, I would like to extend our sincere gratitude for the opportunity to revise our manuscript. We have meticulously addressed the reviewers' comments by carefully revising the manuscript and incorporating new experimental data as recommended. For ease of review, the updated parts have been highlighted in yellow in the revised manuscript. Detailed responses to each of the comments are provided below.

Title: ACC1 knockdown promotes esophageal squamous cell carcinoma metastasis via the CXCL8–NET axis

Authors: Jiaping Tang, Bo Qi, Qingya Zhuo, Shuhua Huo, Xinchen Dang, Tianbao Pang, Kexin Cao and Yuzhen Liu*, Baosheng Zhao*

Manuscript No.: COMMSBIO-25-2391A

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In this study, Tang et al. focused on the function of ACC1 in ESCC metastasis progression of ESCC, in which they identified the key role of ACC1-CXCL8-NETs axis. Generally, the current results provide good support for the conclusion in this study. However, some issues need to be addressed seriously.

1. In the section of “MATERIALS AND METHODSA”, authors stated that IHC was performed based on the tissue microarray (TMA) containing 97 cases of ESCC, however, no relevant results, especially the protein levels of ACC1, were displayed in the manuscript.

Reply: Thank you for pointing out this important issue. In the original manuscript, the immunohistochemistry (IHC) analysis using the ESCC tissue microarray (TMA) was primarily applied to assess NETs-related markers (MPO and citrullinated histone H3), as presented in Figure

8E. The protein expression of ACC1 was not included at that stage, as our initial evaluation of ACC1 expression and its correlation with clinical outcomes was based on RT-qPCR analysis of frozen ESCC tissues. In response to your suggestion, we have performed IHC staining for ACC1 on the same TMA. Due to tissue loss during TMA sectioning and staining, 6 samples were excluded, resulting in a total of 91 cases available for analysis. The corresponding results, including representative images and statistical analysis, have been added to the revised manuscript as Figure 1D–F. This new data provides direct evidence of ACC1 protein expression in ESCC tissues and its association with clinicopathological features, thereby strengthening the translational relevance of our findings. These results have been incorporated in the revised manuscript file (Lines 143–151). We have also clarified the final sample number (91 cases) and the reason for exclusion in the revised manuscript file (Lines 645-647).

2 . In Figure 1A, authors analyzed ACC1 mRNA levels in the group with good or poor prognosis, which missed the detailed standards about the definition about different prognosis. In Figure 1C, the correlation between ACC1 protein levels and clinical prognoses is more worthy of evaluation.

Reply: Thank you for your insightful comment. In the revised manuscript, we have clarified the criteria used to define prognostic subgroups in Figure 1A. Specifically, patients with overall survival (OS) ≥60 months (5 years) were classified as the favorable prognosis group, while those with OS ≤14 months (1.16 years) were categorized as the poor prognosis. The deviation of our poor prognosis definition (OS ≤ 14 months) from the conventionally used threshold (OS ≤ 12 months) was deliberate and driven by considerations of sample size balance and statistical power within our cohort. The rationale is detailed below: Initial stratification using strict criteria (Favorable: OS ≥ 60 months; Poor: OS ≤ 12 months) revealed a significant imbalance in group sizes: the poor prognosis group (OS ≤ 12 months) contained substantially fewer patients than the favorable prognosis group (OS ≥ 60 months). To enhance group comparability, improve statistical power, and increase the reliability of comparisons, we implemented a minimal, justified expansion of the poor prognosis group inclusion criteria. Specifically, the upper limit for poor prognosis was extended from OS = 12 months to OS = 14 months. This adjustment incorporated only 8 additional patients with survival times between >12 and ≤14 months (i.e., 12 < OS ≤ 14) into the poor prognosis group. We contend

that the prognosis for patients surviving 12-14 months remains clinically unfavorable, justifying their classification within the "poor prognosis" category. This adjustment was deliberate and minimal (spanning only 2 months), implemented specifically to address the sample size imbalance while preserving the clinical relevance of the group definitions. The detailed criteria have been added to the Supplementary Materials (Lines 189–208).

We also fully agree that assessing the correlation between ACC1 protein expression and clinical outcomes adds important translational value. As noted in our response to Comment 1, we have now performed IHC staining for ACC1 on the ESCC tissue microarray and conducted Kaplan–Meier survival analysis. As shown in the updated Figure 1D–F, low ACC1 expression is significantly associated with poorer overall survival ($P < 0.001$). In addition, ACC1 protein levels were significantly lower in tumor tissues than in adjacent normal tissues ($P < 0.001$). These results further support ACC1 as a potential prognostic biomarker in ESCC.

3. In Page 13 Line 281, the description about “an independent predictor” should result from the Multivariate Cox Regression analysis.

Reply: Thank you for your correction. We agree that the term "independent predictor" should be based on multivariate Cox regression analyses. Accordingly, we have revised the original statement on Page 13, Line 281 to: “Together, these findings suggest that decreased ACC1 expression is associated with aggressive clinicopathological features and unfavourable survival outcomes in ESCC, supporting its potential as a prognostic biomarker.” (Line 143-151 of revised manuscript file)

4. The scale bar in the images from transwell assays should be indicated clearly.

Reply: Thank you for pointing this out. We have revised the transwell assay images to include a clearly marked scale bar (100 μm), which is now also mentioned in the figure legends. Please see the updated figures in the revised manuscript file.

5. The spelling mistake about “Metastesis” in Figure 7E should be corrected.

Reply: Thank you for pointing out this typographical error. The misspelling of “Metastesis” in Figure 7E has been corrected to“Metastasis”in the figure panel. The revised version of Figure 7E has been included in the revised manuscript file.

6. The grammar in this manuscript needs to be carefully proofread.

Reply: Thank you for your helpful comment. In response, we have had the entire manuscript professionally edited by Editage to ensure clarity, consistency, and grammatical accuracy. All language-related issues have been addressed accordingly, and the certificate of editing is provided along with the revised submission (Line 523 of supplemental materials).

Reviewer #2 (Remarks to the Author):

Dear Author,

I sincerely appreciate the opportunity to review this manuscript. In the present study, the authors focus on the role of Acetyl-CoA carboxylase 1 (ACC1) in esophageal squamous cell carcinoma (ESCC). When ACC1 knockdown in ESCC cells, it promotes epithelial-mesenchymal transition (EMT) and enhances cell migration and invasion by activating the PI3K/AKT and MEK/ERK pathways through CXCL8 up-regulation. I think this research is well written and the experimental design is reasonable. However, there are some faults that the authors need to amend and explain. For example, in Page 9, "Western blot" section, after PVDF membranes transferred, the PVDF membranes need be blocked with fat-free milk or BSA. Furthermore, the authors claim that "Total protein of 20 μg for each sample was separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) ..." in Page 9, but the images of Western blot look like something else. For example, in Fig. 4, the "beta-actin" protein bands in KYSE 150 and 450 don't like the images of Fig. S2 A & B and other Figures in "Original western blots". Therefore, this manuscript should be carefully revised to be suitable for publication.

Reply: We sincerely thank the reviewer for the careful reading of our manuscript and the valuable suggestions.

1. Regarding the Western blot

   We apologize for the omission in the Methods section. As correctly pointed out, after transferring the proteins onto PVDF membranes, we blocked the membranes with 5% fat-free milk diluted in TBST (Tris-buffered saline with Tween-20) for 1 hour at room temperature

before incubation with primary antibodies. This information has now been added to the revised manuscript file (Lines 585-586) for completeness and clarity.

2. **Regarding the concern about Western blot image consistency**

   We appreciate your detailed observation regarding the β-actin protein bands in Fig. 4I, Fig. S2A/B, and the original Western blot images. Although equal amounts of total protein (20 μg) were loaded in all experiments, the visual intensity of β-actin bands may vary slightly across different figures due to differences in exposure time or imaging settings used during blot development. This variation does not affect the internal consistency within each experiment, where all comparisons were performed using blots developed under identical conditions. To address your concern more directly, we have:

   1) Replaced the β-actin panels in Fig. 4I and Fig. S2A/B with more appropriately exposed images from the original data to better reflect uniform loading.
   2) Corrected the previous mislabeling of the original blot for Fig. 4I, which had been mistakenly marked as "Fig. 3I" in the supplemental materials.
   3) Carefully re-examined all Western blot figure labels and their corresponding raw data to ensure accuracy and consistency across the manuscript.

   We have also added a clarification in the revised manuscript to explain that representative images were selected from independent experiments and developed under varying imaging conditions (Lines 595-600).

Reviewer #3 (Remarks to the Author):

The paper identifies a new mechanism by which ACC1 knockdown promotes esophageal squamous cell carcinoma (ESCC) metastasis through the CXCL8-NETs axis. It demonstrates that ACC1 expression is associated with poor prognosis in ESCC patients and that ACC1 knockdown enhances ESCC cell migration and invasion in vitro. Furthermore, it verifies the role of the ACC1-CXCL8-NETs axis in ESCC metastasis using xenograft tumor models and lung metastasis models. The topic is interesting and the data analysis is convincing.

The Minors Concerns:

1. It would be more compelling to use immunohistochemical analysis for testing the prognostic value of ACC1 in ESCC patients.

Reply: Thank you very much for the suggestion. We fully agree that evaluating ACC1 expression at the protein level in clinical samples is important to validate its prognostic significance. In response, we have performed immunohistochemical (IHC) staining for ACC1 using a tissue microarray (TMA) comprising ESCC patient samples. The IHC results, now included in Figure 1D–F, show that low ACC1 protein expression is significantly correlated with poorer overall survival ($P < 0.001$) and ACC1 protein expression was significantly lower in tumor tissues compared to adjacent normal tissues ($P < 0.001$), thereby supporting its potential role as an adverse prognostic biomarker in ESCC.

2. While the study shows that ACC1 knockdown promotes ESCC metastasis via the CXCL8-NETs axis, the specific molecular mechanisms underlying ACC1's regulation of CXCL8 require further exploration. For example, are there other transcription factors or signaling pathways involved in addition to c-Fos?

Reply: We thank the reviewer for this insightful and constructive comment. We agree that CXCL8 expression is regulated by a complex transcriptional network and may involve additional mechanisms beyond c-Fos. In our study, luciferase reporter assays and ChIP-qPCR confirmed that c-Fos directly binds to and activates the CXCL8 promoter (Figures 4C, D). Functional assays further demonstrated that c-Fos knockdown abolished ACC1 loss-induced CXCL8 upregulation and enhanced ESCC cell migration/invasion (Figure 4F–H), indicating that c-Fos is a central and indispensable mediator in this axis.

To further address the reviewer’s question, we have examined our RNA-seq data (available in Figshare, with the link: 10.6084/m9.figshare.27173397) and found that two additional CXCL8-associated transcription factors, JunB and C/EBPβ, were also significantly upregulated upon ACC1 knockdown, and this upregulation was confirmed by qPCR (Supplemental Figure 5G). These factors are known to regulate CXCL8 through AP-1 or C/EBP motifs, respectively. Although not functionally validated in this study, their consistent induction suggests that ACC1 knockdown may engage a broader transcriptional regulatory network to activate CXCL8.

These insights have been incorporated in the revised manuscript file (Lines 455–461). We appreciate the reviewer's suggestion, which has helped us strengthen the mechanistic depth of the manuscript. In future work, we plan to further investigate whether JunB and C/EBPβ directly contribute to CXCL8 upregulation in the context of ACC1 knockdown, using targeted knockdown and ChIP-based approaches.

3. The author should explore the therapeutic potential of targeting the ACC1-CXCL8-NETs axis by evaluating the effects of CXCL8 inhibitors or NETs inhibitors on ESCC metastasis in preclinical models.

Reply: We thank the reviewer for this important suggestion. As part of our original submission, we had already performed in vivo therapeutic intervention experiments using PAD4i, a well-characterized PAD4 inhibitor that effectively blocks NETs formation. As shown in the manuscript (Figure 7), PAD4i treatment in the ESCC xenograft metastasis model markedly reduced NETs formation and significantly suppressed lung metastasis induced by ACC1 knockdown. These findings provide direct preclinical evidence supporting the therapeutic potential of targeting NETs to counteract ACC1 loss-driven ESCC metastasis. Additionally, we conducted in vitro co-culture experiments in which treatment with either a CXCL8-neutralizing antibody or CXCR1/2 inhibitor significantly attenuated the pro-metastatic effects of ACC1-deficient ESCC cells (Figure 2F, G; Figure 3B-E). These results further support the ACC1–CXCL8–NETs axis as a promising therapeutic target. However, we acknowledge that in vivo validation using CXCL8 or CXCR2 inhibitors was not performed in this study, and we now clearly state this limitation in the revised manuscript file (Lines 509-514). We plan to address this important question in future studies to more fully evaluate the translational potential of pharmacologically targeting CXCL8 signaling in ESCC.

4. The author should analyze the correlation between the expression of relevant molecules and clinical outcomes in a larger cohort of ESCC patients to validate the clinical efficacy of these therapeutic targets.

Reply: Thank you for this suggestion. In our current study, we analyzed a cohort of 91 ESCC patients with available clinical data and found that low ACC1 expression significantly correlates with worse overall survival, and is accompanied by increased CXCL8 expression and elevated NETs

formation (Figure 8H). Furthermore, Kaplan–Meier support the prognostic significance of this axis. However, we fully agree that validation in a larger and more diverse patient cohort is critical to further confirm the clinical relevance of targeting the ACC1–CXCL8–NETs axis. To address this limitation, we are currently expanding our clinical sample size, integrating longitudinal survival data, and applying multi-omics approaches to further explore the heterogeneity and translational potential of this pathway in ESCC progression. This ongoing effort has also been added to the revised manuscript file (Lines 520-524) as a limitation and future direction.

Chief Editor
Communications Biology
September 26, 2025

Dear Editor,
Thank you for forwarding the final comments from the reviewers. We are delighted that the reviewers are satisfied with our revisions and that our manuscript is now one step closer to publication.

Title: Acetyl-CoA carboxylase 1 knockdown promotes esophageal squamous cell carcinoma metastasis via the CXCL8–NET axis
Authors: Jiaping Tang, Bo Qi, Qingya Zhuo, Shuhua Huo, Xinchen Dang, Tianbao Pang, Kexin Cao and Yuzhen Liu*, Baosheng Zhao*
Manuscript No.: COMMSBIO-25-2391B

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The issues have been appropriately resolved in the revised manuscript. However, the explanation about scale bars in the images from transwell assays was still not found in the figure legends of Figs.2-6.

Reply: We sincerely thank Reviewer #1 for the positive feedback and for drawing our attention to this oversight. In the revised manuscript, the figure legends for Figures 2–6 have been updated to explicitly include the scale bar information for all transwell assay images. We apologize for the previous omission and appreciate the reviewer's meticulous comments, which have helped enhance the clarity and completeness of our work.

Reviewer #2 (Remarks to the Author):

Dear Authors,

In the present study, the authors focus on the role of Acetyl-CoA carboxylase 1 (ACC1) in esophageal squamous cell carcinoma (ESCC). When ACC1 knockdown in ESCC cells, it promotes epithelial-mesenchymal transition (EMT) and enhances cell migration and invasion by activating the PI3K/AKT and MEK/ERK pathways through CXCL8 up-regulation. I think this research is well written and the experimental design is reasonable. The authors also revise the manuscript according to the reviewers' comments. Therefore, I suggest that this manuscript could be published in this journal.

Reply: We sincerely thank Reviewer #2 for the encouraging comments and the recommendation for publication. We appreciate the careful review and are pleased that our revisions have addressed the previous concerns and that the manuscript was considered well-written with a reasonable experimental design.