**Peer Review File**

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**Reviewer A**

Xie et al., investigated the associated of high expression of lncRNA GAS6-AS1 with the lymph node metastasis in esophageal squamous cell carcinoma (ESCC). This research stand with novelty and useful to further understand the involvement of lncRNA as a predictive indicator or biomarker, particularly in the lymph node metastasis of ESCC. The manscript writing and organization are acceptable. However, some points are required to be revised before acceptance for publication:

1. Title – the current title does not reflect the content of the manuscript. The current title indicates that the lymph node metastasis-related GAS6-AS1 facilities progression of ESCC.
Reply 1: Thank you for your comments concerning our manuscript, firstly, our manuscript mainly investigated the associated of GAS6-AS1 and lymph node metastasis (see Page 2, line 14-16). Secondly, our manuscript found that high expression of GAS6-AS1 was related to poor tumor differentiation, tumor-node-metastasis (TNM) staging, T staging, and lymph node metastasis (see Page 3, line 8-11). Thirdly, high expression of GAS6-AS1 was related to poor prognosis (see Page 3, line 13-15), what's more, GAS6-AS1 knockdown inhibited the proliferation, colony formation, cycle and induced apoptosis of ESCC cells, which all indicated lncRNA GAS6-AS1 facilitates the progression of esophageal squamous cell carcinoma (see Page 3, line 19-20).
Changes in the text: we have modified our text as advised (see Page 2, line 14-16)/ (see Page 3, line 8-11)/ (see Page 3, line 13-15)/ (see Page 3, line 19-20).

2. English language – some English grammar and a little sentence structure mistakes have been found. Kindly double check during revision stage.
Reply 2: Thank you for your advice, we have kindly checked during revision stage.
Changes in the text: we have modified our text as advised (see Page6, line10-15)/ (see Page6, line10-15)/ (see Page6, line10-15)/ (see Page6, line10-15)/ (see Page8, line3-7)

3. Method – the description and information about each method should be detailed in order to ensure the data is reproducible and as a reference to other researchers in the related field. Specifically, for "Cell Lines" method, HET-1A should be esophageal normal epithelial cell line, thus should not group under ESCC. Beside, for "Xenograft Tumor Model", is the medium RPMI-1640 or DMEM?

Reply 3: Thank you for your comments, HET-1A was esophageal normal epithelial cell line (see Page7, line11-13). And for "Xenograft Tumor Model", is the medium RPMI-1640 (see Page9, line8), we have corrected the error accordingly.
Changes in the text: we have modified our text as advised (see Page7, line11-13)/(see Page9, line8 )

4. Result – it is not recommended to cite in this section. Please check.
Reply 4: Thank you for your reminder, we have deleted some references.
Changes in the text: we have modified our text as advised (see Page10, line17-18).

5. Additional references – the authors should cite important publications like https://www.mytopscientists.org/v3/info/RBS.aspx and https://doi.org/10.1155/2021/5519720
Reply 5: Thank you for your recommendation, we have cited this paper.
Changes in the text: we have modified our text as advised (see Page4, line23-25).

**Reviewer B**

The useful sources:
https://doi.org/10.1007/s12253-019-00711-3
https://doi.org/10.17179/excli2018-1847
https://doi.org/10.1007/s11033-018-4556-2
PMCID: PMC3558201
Reply: Thank you for the reviewers' comments concerning our manuscript, we have cited these important papers.
Changes in the text: we have modified our text as advised (see Page14, line9-10) (see Page14, line17-18) (see Page5, line5-7) (see Page4, line16-18).

**Reviewer C**

The paper titled "Lymph node metastasis-related lncRNA GAS6-AS1 facilitates the progression of esophageal squamous cell carcinoma" is interesting. The results revealed that lncRNA GAS6-AS1 obtained from RNA-seq can be used as an independent risk factor for ESCC lymph node metastasis and an effective biomarker to predict ESCC, and that it was related to the growth and metastasis of ESCC. It may represent a new biomarker to aid in the assessment of the lymph node metastasis of ESCC. However, there are several minor issues that if addressed would significantly improve the manuscript.
1) There have been many studies on ESCC. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply 1: Thank you for your comments concerning our manuscript, our difference between this study and previous studies and our innovation: our study reported the comprehensive sequencing of transcriptome in ESCC tissues with or without lymph node metastasis in T3 phase by using RNA-seq for the first time. We have described in the introduction (see Page6, line12-15).
Changes in the text: we have modified our text as advised (see Page6, line12-15).

2) Many boxes in the figures are incomplete and the letter markings are not uniform. Please carefully check the figures and make corrections.
Reply 2: Thank you for your comments, we have carefully checked the figures and make corrections.

3) Can lncRNA GAS6-AS1 be used as a potential biomarker for patient risk stratification and local regional metastasis in ESCC? It is recommended to add relevant content.
Reply 3: lncRNA GAS6-AS1 can be used as a potential biomarker for patient risk stratification and local regional metastasis in ESCC, we add relevant content (see Page13, line4-5).
Changes in the text: we have modified our text as advised (see Page13, line4-5).

4) How to determine the predictive factors of lymph node metastasis in ESCC? It is recommended to add relevant content.
Reply 4: The predictive factors for ESCC lymph node metastasis should have certain sensitivity and specificity, and we used ROC curves to evaluate predictive value (see Page10, line1).
Changes in the text: we have modified our text as advised (see Page10, line1).

5) Suggest increasing the analysis and research of ceRNA, which may make the entire experiment more complete.
Reply 5: Thank you for your comments, we have increased the analysis and research of ceRNA (see Page17, line2-4), and studying the specific signaling pathway of GAS6-AS1 affecting lymph node metastasis of ESCC is our next research plan.
Changes in the text: we have modified our text as advised (see Page17, line2-4).

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Microarray analysis of miRNA based on the regional lymph node metastasis status of esophageal squamous cell carcinoma, Transl Cancer Res, PMID: 35116259”. It is recommended to quote the article.
Reply 6: Thank you for your recommendation, we have cited this important paper (see Page15, line3-5).
Changes in the text: we have modified our text as advised (see Page15, line3-5).

7) It is suggested that the research progress of lncRNA in ESCC should be added to the discussion.

Reply 7: We have added the research progress of lncRNA in ESCC to the discussion (see Page14, line20-23).

Changes in the text: we have modified our text as advised (see Page14, line20-23).

8) What is the impact of this study on the further treatment and prognosis of ESCC? It is recommended to include relevant content in the discussion.

Reply 8: The impact of this study on the further treatment and prognosis of ESCC: The lncRNA GAS6-AS1 can be used as an independent risk factor for ESCC lymph node metastasis and an effective biomarker for its prediction, which may highlight as a promising oncogene of clinical value (see Page16, line17-20).

Changes in the text: we have modified our text as advised (see Page16, line17-20).

**Reviewer C**

**1. Reference/citation**

The authors mentioned "studies...", while only one reference was cited. Change "Studies" to "A study/A previous study" or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

*Previous studies have shown that the expression of lncRNA BANCR gene significantly increased in tumor tissue, and overexpression of lncRNA BANCR was positively correlated with lymph node metastasis (18).*

*Some studies have shown that overexpression of GAS6-AS1 can inhibit tumor progression of lung adenocarcinoma (LUAD) both in vivo and in vitro, which is also associated with adverse clinical prognosis (20).*

*On the contrary, studies have shown that the high expression of GAS6-AS1 is closely related to the tumor staging of gastric cancer, and in vitro and in vivo experiments have shown that it can promote tumor growth, metastasis, and cell cycle changes (19).*

*More studies have shown that this lncRNA can serve as a sponge for miR-24-3p (26) to regulate GIMAP6. Therefore, the interaction between GAS6-AS1 and microRNA (miRNA) is the most likely pathway for GAS6-AS1 to participate in tumor development.*

*Some studies suggest that lncRNA can serve as a potential biomarker, such as lncRNA PCAT-1, may consider as a candidate prognostic biomarker for ESCC (38).*

*Subsequent studies showed that knocking down GAS6-AS1 reduced cell proliferation, …, which are consistent with those of studies on other tumors (19).*

Reply: We have revised, changed "Studies" to "A study/A previous study" or add more citations.

**2. Figure 1A and Figure 3(A, B)** should be cited as table in the text, and they should be cited consecutively in the text and numbered in the order in which they are discussed. Tables must be typed and editable in a tabular format that is convenient for copyediting and typesetting; the preferred format is doc. Each table must **include** the table title, appropriate column heads, and explanatory legends (including definitions of any abbreviations used).

Please refer to the example (https://cdn.amegroups.cn/static/public/3.9-Table-examples.pdf) for different cases.

a. Please also add a table header in figure 1A.

A

| |
|---|
| Gender |
| Age (years) |
| Pathological Types |
| Differentiation grade |
| Primary tumor invasion depth |
| TNM stage |
| Number of lymph node dissection |
| Lymph node metastasis |

b. Figure 3A

Please indicate the P value in the red box.

A

| Clinical parameters | High expression n=93 | Low expression n=94 | *P* value |
|---|---|---|---|
| Gender | | | |
| Male | 41 | 54 | 0.068 |
| Female | 52 | 40 | |
| Age (years) | | | |
| < 60 | 19 | 24 | 0.407 |
| ≥60 | 74 | 70 | |
| Differentiation grade | | | |
| Poor | 40 | 26 | **0.028** |
| Well/moderate | 53 | 68 | |
| TNM stage | | | |
| I/II stage | 33 | 66 | **<0.001** |
| III/IV stage | 60 | 28 | |
| Primary tumor invasion depth | | | |
| T1/T2 | 35 | 54 | **0.007** |
| T3/T4 | 58 | 40 | |
| Lymph node metastasis | | | |
| Negative | 31 | 65 | **<0.001** |
| Positive | 62 | 29 | |

c. Figure 3B

Please revise “CI” to “95% CI”.

B

| Clinical parameters | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | HR | CI | *P* | HR | CI | *P* |
| Gender | 0.98 | 0.553-1.74 | 0.46 | - | - | - |
| Age | 1.005 | 0.978-1.034 | 0.71 | - | - | - |
| Differentiation | 3.884 | 2.045-7.378 | **<0.001** | 3.651 | 1.826-7.302 | **<0.001** |

Reply: We have made the necessary modifications as required.

3. Since Figure 1A and Figure 3(A, B) should be cited as table, please update the order of figures in the main text, and revise the letter (A, B, C,…) in figures (1, 3) and figure legends. Please also check the abbreviations in the figure legends. For not included abbreviations, please remove their definitions.

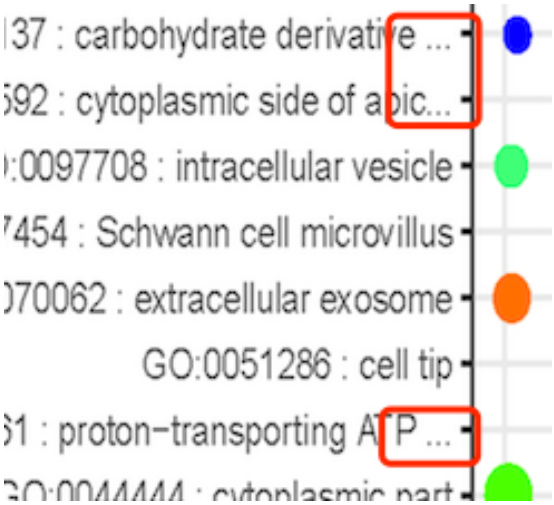
Reply: We have updated the order of figures in the main text, and revised.

4. Figure 1D is not clear enough for publication. It would be much appreciated if you could provide it with a higher resolution as possible as you could. The preferred format is JPG or TIFF.

Reply: We have provided it with a higher resolution

**5. Figure 1G, H, I, J**

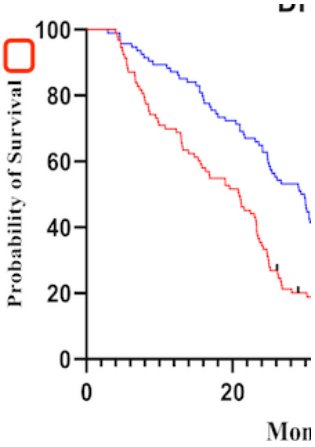
It seems that some words are incomplete, please check and revise.




Reply: We have checked and revised.

**6. Figure 2E, F, G, H**

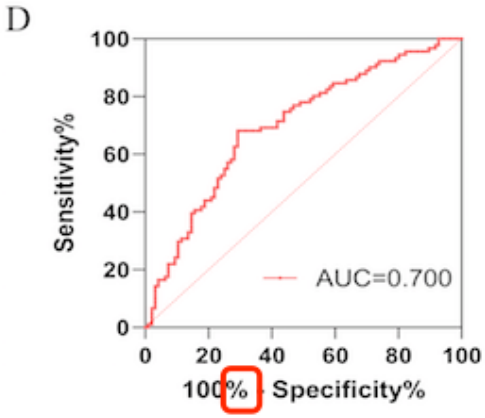
Please add a unit (%) in the Y-axis.




Reply: We have made the necessary modifications as required.

**7. Figure 3D**

Please deleted the "%" we pointed out.




Reply: We have made the necessary modifications as required.

**8. Figure 4F, G**

Please indicate the observation method in the figure legend.

730 (KYSE410/TE-1). (F,G) The colony formation assays were performed in GAS6-AS1
731 stable knockdown and control cells (TE-1/KYSE410) to detect the effect of growth
732 (crystal violet staining, ×200). (H,I) Wound scratch assay analysis of knockdown of

Reply: We have indicated the observation method in the figure legend.

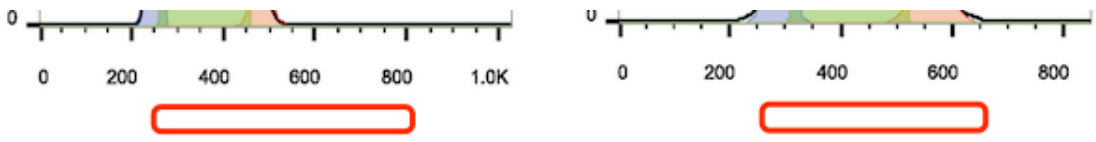
**9. Figure 5**

No '**' in figure 5, but it was explained in figure legend. Please check and revise.


Reply: We have deleted it.

**10. Figure 5C, D**

Please provide a description in the X-axis.



Reply: We have provided a description in the X-axis..

11. Please indicate which figure. Is it figure 4? Please note that Figures should be cited consecutively in the text and numbered in the order in which they are discussed. (example: Figure 1 contains 4 parts, such as Figure 1A, 1B, 1C, 1D, these parts should also be cited consecutively, unless Figure 1 is already cited before Figure 1A, 1B, 1C, 1D.)

236 total of three separate fields were photographed for each plate (1 representative image
237 is shown in the figure). [illegible] wound healing assay was conducted using a 200 µL pipette

Reply: We have revised.