TCR TRANSLATIONAL CANCER RESEARCH
ADVANCES CLINICAL MEDICINE TOWARD THE GOAL OF IMPROVING PATIENTS' QUALITY OF LIFE
IMPACT FACTOR 1.241

Peer Review File

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Reviewer A

The manuscript represents an analysis of regulatory RNA pathways in adenoid cystic carcinoma.

The following are the main issues in this study:

1. The rationale and relevance of selecting these pathways in ACC are unclear. The mere lack of previous studies of these molecules is unconvincing justification.

Reply 1: Thanks for your valuable comment. In our study, we obtained RNA sequencing data from the Gene Expression Omnibus (GEO) database. We screened differentially expressed RNAs by Edge R package and gained functional enrichment analysis of DE-mRNAs, meanwhile, we also constructed the lncRNA-miRNA-mRNA regulatory network and PPI network. According to lncRNA-miRNA-mRNA regulatory network, NONHSAT251752.1 NONHSAT232163.1, NONHSAT250051.1, NONHSAT192459.1 are the top 4 nodes with the highest degree in regulatory networks and were screened out and visualized by using the Cytoscape software. Then, we screened lncRNA-miRNA-mRNA regulatory axis based on NONHSAT251752.1 NONHSAT232163.1, NONHSAT250051.1, NONHSAT192459.1. And NOTCH1, IGF1, CDK6 play important roles in ACC according to literature research. Moreover, in PPI network, circles represent mRNA, the larger the dot, the stronger the regulation of mRNA. NOTCH1, IGF1, CDK6 have the largest dot, which verifies the accuracy of the regulatory axis we screened. Finally, through PCR verification, we determined NONHSAT251752.1-hsa-miR-6817-5p-NOTCH1, NONHSAT251752.1-hsa-miR-204-5p/hsa-miR-138-5p-CDK6 as the key regulatory axise. However, at present, the lncRNA-miRNA-mRNA network was not verified through further research, which is also a great deficiency of this article. In the future, we will verify it with more experiments.

2. No functional and/or experimental validation of selected targets in cell lines and/or PDX models are performed. Without this data, the significance of the findings are

doubtful.

Reply 2: In our study, our focus is to find the significantly upregulated and downregulated RNA, pathways, lncRNA-miRNA-mRNA network in SACC through bioinformatics analysis, and verify the reliability of bioinformatics analysis by PCR. So our aim is to provide a basis for the study of ceRNA in SACC. At present, there is no acknowledged ACC cell line in database. According to literature reports, it is commonly used internationally six ACC cell lines, ACC2, ACC3, ACCM, ACC-NS, ACCs and CAC2 have cross contamination and misidentification. For the domestic ACC cell line SACC-83, it is still uncertain whether there are problems such as cross contamination or wrong identification. Thank you for the PDX model proposed by the teacher, we will focus on your suggestions. However, the construction of PDX model in ACC is not mature yet. It is difficult to build and the success rate is low. The detailed regulation effect and underlying mechanism of the regulatory axis still need further investigation and it would be our focus in future work.

3. The status of selected RNA Molecules in primary should be performed.

Reply 3: In our study, we determined NONHSAT251752.1-hsa-miR-6817-5p-NOTCH1, NONHSAT251752.1-hsa-miR-204-5p/hsa-miR-138-5p-CDK6 as the key regulatory axise. However, we just analyze the current literature research without further experimental verification, which is a great deficiency. In the subsequent experiments, we will deeply explore the role of ceRNA in SACC.

Reviewer B

This is a fascinating study about the role of competitive endogenous RNA in adenoid cystic carcinoma of the salivary gland. There is no study in the literature regarding this topic. The authors concluded that long ncRNAs might play a critical role in the SACC pathogenesis. This study adds new and important information to the literature; however, some points should be highlighted:

1. Keywords:

(a) We recommend the use of Medical Subject Headings (MeSH-NCBI). There are no MeSH terms such as “bioinformatics analysis”, “competitive endogenous RNA,” and

"lncRNA-miRNA-mRNA network". Maybe it is more enjoyable to use "Salivary gland Neoplasm" instead of "adenoid cystic carcinoma." This can simplify other authors' findings of your article.

Reply 1: The Keywords has been revised according to the comment.

Changes in the text: We have modified our text as advised (see line 65).

2. Introduction:

(a) the First paragraph of the introduction: "Adenoid cystic carcinoma of the salivary gland (SACC) is one of the most common salivary gland carcinomas, accounting for 1% (1) of head and neck malignant tumors and 30.42% (2) of all malignant tumors of the salivary gland." The study the authors cited is based on the Chinese population only.

According to WHO 2017 (El-Naggar AK, et al. World Health Organization Classification of Tumours: Pathology and Genetics of Head and Neck Tumours, 4th ed. Lyon, France: International Agency for Research on Cancer (IARC); 2017.), ACC accounts for < 1% of head and neck cancers and < 10% of salivary gland neoplasms.

Reply 2: "Adenoid cystic carcinoma accounts for 30.42% of all malignant tumors of the salivary gland."

This is a result from the literature "Salivary gland neoplasms in oral and maxillofacial regions: a 23-year retrospective study of 6982 cases in an eastern Chinese population." It is reported by Shanghai Ninth People's Hospital based on eastern Chinese population. This similarity in the frequencies differed from some reports from Brazil, Jordan, Congo, Iran and Tanzania. So we change the content into classical conclusion "Adenoid cystic carcinoma (ACC) is a relatively rare cancer, accounting for 1% of head and neck tumors and 10% of salivary gland tumors," from the literature "Spiro RH, Huvos AG, Strong EW. Adenoid cystic carcinoma of salivary origin - clinicopathologic study of 242 cases. American Journal of Surgery. 1974;128(4):512-20"

Changes in the text: We have modified our text as advised (see line 67-68).

3. Results:

(a) Figure 2. What are the parameters utilized for building the heatmap? What was the distance used (Eg. Euclidian, Manhattan).

Reply 3: Heatmap is built by the parameters of $P < 0.05$ & $FDR < 0.05$ & $FC > 2$ and we use Euclidian as the distance.

Changes in the text: We have modified our text as advised (see line 533-534).

4. Discussion:

(a) The results are exciting; however, the discussion is poor, for example, in figure 4. B the most enriched pathway was the metabolic processes, this is a result significant, explain more about the role of metabolism in SACC (doi:10.1111/cpr.12705).

Reply 4: Thank you for your comment, and thank you for your recognition of the results. Among the pathways that were down-regulated in our bioinformatics analysis, the metabolic pathway was significantly down-regulated, which predicts that the metabolic pathway plays an important role in the occurrence and development of ACC. In the literature of "PRRX1−induced epithelial−to−mesenchymal transition in salivary adenoid cystic carcinoma activates the metabolic reprogramming of free fatty acids to promote invasion and metastasis", metabolism is closely related to epithelial mesenchyme. The role of metabolism in ACC is worthy of in-depth discussion, and the current mechanism is not yet perfect.

Changes in the text: We have modified our text as advised (see line 282-286).

(b) The results of figure 7. E is exciting. Explain more about SOX4, a down-regulated mRNA in Figure 6, and its role in the SACC. (doi:10.1038/sj.onc.1209566).

Reply5: Thank you for your comment. According to the P value, PPI network, lncRNA- miRNA-mRNA network, and the literatures, only a small part of lncRNA-miRNA-mRNA network is reported. The manuscript has been revised accordingly.

Changes in the text: We have modified our text as advised (see line 286-289).

5. Conclusion:

(a) The authors may be more concise and objective.

Reply 6: The conclusion has been revised according to the comment.

Changes in the text: We have modified our text as advised (see line 336-343).

6. Materials Design Analysis Reporting (MDAR) Checklist for Authors:

(a) The authors do not provide (1) sample size determination; (2) Inclusion/exclusion criteria; (3) statistical tests used and justify the choice of tests that needed a more robust explanation.

Reply 7: (1) sample size determination: 3 samples for PCR were used in our study.

(2) Inclusion/exclusion criteria: These patients met the following inclusion and exclusion criteria: the tumor was primary, the pathological type was ACC, patients with preoperative radiotherapy or chemotherapy were excluded, patients with other malignant tumors were excluded.

(3) statistical tests used and justify the choice of tests that needed a more robust explanation: Most of statistical analysis was carried out through corresponding databases mentioned above, and the rest of statistical analysis was performed by using R software. The Student's t-test was used to evaluate the differences of mRNAs, miRNAs and lncRNAs expression between cancer and normal samples. Pathway enrichment analysis was estimated by Fisher's test, and false discovery rate (FDR) was calculated to correct the p values. The results of RT-qPCR were repeated three times for the three matched samples. Data were presented as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 8.0.2 (GraphPad Software, USA). Differences between the two groups were determined by T-test. $P<0.05$ was considered statistically significant.

Changes in the text: We have modified our text as advised.