Peer Review File

Article Information: https://dx.doi.org/10.21037/atm-23-30

Reviewer A

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide and one of the leading causes of cancer death, with an estimated 600,000 people living with HNSCC every year. In the manuscript “The lncRNA XIST/miR-29b-3p/COL3A1 axis regulates central carbon metabolism in head and neck squamous cell carcinoma and is associated with poor tumor prognosis”, authors investigated the association between head and neck squamous cell carcinoma (HNSCC) and Collagen Type III Alpha 1 Chain (COL3A1). Couple questions are required to be answered before it will be accepted.

(1) In the background of abstract, the aim of the study was “to investigated the association between HNSCC and COL3A1”. This summary was incomplete.

Answer: We have adjusted the " background " part of the abstract to " Recent evidence shows that COL3A1 promotes the progression of many types of cancer. The purpose of our study is to explore the correlation between COL3A1 and the prognosis of patients with head and neck squamous cell carcinoma and its potential mechanism." See the red part: page1-2, line32-35.

(2) What were the roles of COL3A1 in the tumorigenesis and development of HNSCC? Please state in the introduction.

Answer: We have added relevant contents and corresponding references in the preface. “At present, there are few related studies on the role of COL3A1 in HNSCC. One study reported that COL3A1 has a good role in evaluating the immune cell infiltration, immune activity and gene expression of immune checkpoint of HNSCC lesions" [Shen Y, Li X, Wang D, Zhang L, Li X, Su L, Fan X, Yang X. COL3A1: Potential prognostic predictor for head and neck cancer based on immune-microenvironment alternative splicing. Cancer Med. 2022. doi: 10.1002/cam4.5170.]。See the red part: page4, line120-123.

(3) It was proposed to add related reference (DOI: 10.21037/atm-22-1143) about the prognostic biomarkers in HNSCC.

Answer: We have added the relevant content in the preface: "At the same time, there are many relevant studies to explore the biomarkers for the diagnosis and treatment of HNSCC, such as the identification of SERPINE1, PLAU and ACTA1 as biomarkers of head and neck squamous cell carcinoma based on comprehensive bioinformatics analysis. [Yang K, Zhang S, Zhang D, Tao Q, Zhang T, Liu G, Liu X, Zhao T. Identification of SERPINE1, PLAU and ACTA1 as biomarkers of head and neck squamous cell carcinoma based on integrated bioinformatics analysis. Int J Clin Oncol. 2019; 24: 1030-41. doi: 10.1007/s10147-019-01435-9.]; LINC00958 and HOXC13-AS as key candidate biomarkers of head and neck squamous cell carcinoma

through comprehensive bioinformatics analysis [Xiong D, Wu W, Kan L, Chen D, Dou X, Ji X, Wang M, Zong Z, Li J, Zhang X. LINC00958 and HOXC13-AS as key candidate biomarkers in head and neck squamous cell carcinoma by integrated bioinformatics analysis. PeerJ. 2020; 8: e8557. doi: 10.7717/peerj.8557.] ; CD247 is an independent protective factor for the prognosis of HNSC patients. Promote interferon by activating hsa04650 and hsa04660 pathways γ、 The expression of interleukin (IL) - 2 and IL-10 can improve the body's tumor immune monitoring ability, induce tumor cell apoptosis and inhibit tumor cell growth. [Lin P, Hu XL, Hu YY, Liu MY, Wang QY, Ding Y, Ye JC. Prognostic value of CD247 in patients with head and neck squamous cell carcinoma: bioinformatic analysis of TCGA database. Ann Transl Med. 2022; 10: 923. doi: 10.21037/atm-22-1143.]. See the red part: page3, line 87-96.

(4) Why to focus on COL3A1 in the study? Please state in the introduction.

Answer: COL3A1, as a type III collagen, mainly exists in the extracellular matrix, and is rich in human skin, vascular intima, muscle and other connective tissues. We also supplemented the relevant research reports of COL3A1 in HNSCC. At the same time, we added the relevant content in the preface: "At the same time, the head and neck are rich in connective tissue, so it is also of anatomical significance to study the role of COL3A1 in HNSCC." See the red part: page4, line123-124.

(5) How to construct the nomogram? Please state in the methods.

Answer: We have supplemented and changed relevant contents in the methodology section "In order to personalize the predicted survival probability of 1, 3 and 5 years, we built a nomogram based on the results of multivariate analysis. The RMS R package is used to generate the nomogram, including the clinical features related to COL3A1 and the calibration chart. Calibration and discrimination are the most commonly used methods to evaluate the performance of the model. In this study, the calibration curve is graphically evaluated by mapping the predicted probability of the nomogram and the observed ratio. The 45 degree line represents the best prediction Measured value. The consistency index (C-index) is used to determine the discrimination of the nomogram, which is calculated by the bootstrap method with 1000 resamples. In addition, the prediction accuracy of the nomogram and individual prognostic factors was compared using the C index. " See the red part: page5, line148-158.

(6) There were many miRNAs targeting COL3A1. Why to focus on miR-29b-3p? please state in the results.

Answer: We have explained and supplemented the results, The content was changed to "At the same time, we found that hsa-miR-29b-3p was negatively correlated with COL3A1 in the Starbase database (Figure 8). Based on competitive endogenous RNA (ceRNA) The hypothesis suggests that lncRNA competitively combines with tumor suppressive miRNA to reduce the inhibitory effect of miRNA on target mRNA. Therefore, there should be a negative correlation

between mRNA and target miRNA. Combined with ceRNA hypothesis, survival analysis and correlation analysis, we determined that hsa-miR-29b-3p is the best candidate miRNA. " See the red part.: page8, line251-257.

(7) Missing experimental data was the biggest short board in the study. It was necessary to validate the expression of COL3A1 in HNSCC. It was better to validate the XIST/miR-29b-3p/COL3A1 axis by experiments.

Answer: Thank you very much for your valuable comments. The main purpose of this study is to quickly obtain a mechanism axis that may participate in the occurrence and development of biological events in HNSCC through the method of bioinformatics analysis. At the same time, it is verified and analyzed through the relevant database. In the future research, we will add relevant in vitro and in vivo experiments for further verification and analysis, hoping to provide more reference opinions for the study of the pathogenesis of HNSCC. It also provides more clinical reference value for the treatment of HNSCC patients in the future.

(8) Figure 7 was not clear. Please change it to a higher-resolution one.

Answer: We have replaced the pictures with higher definition.

Reviewer B

1. The authors should also state that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013),

Answer: It has been modified according to the editing requirements.

2. Please indicate the full name of "HNSCC" below. All abbreviated terms should be full when they first appear.

38 **Methods:** We initially screened the differentially expressed gene COL3A1 in The
39 Cancer Genome Atlas (TCGA) database, and the association between the expression
40 level of COL3A1, prognosis, and the clinical parameters of HNSCC patients was

Reply: We have indicated the full name of "HNSCC".

3. Please check if any more references need to be added in the below 5 sentences since you mentioned "Studies", but only one reference was cited. If not, "studies" should be changed to "a study/a previous study".

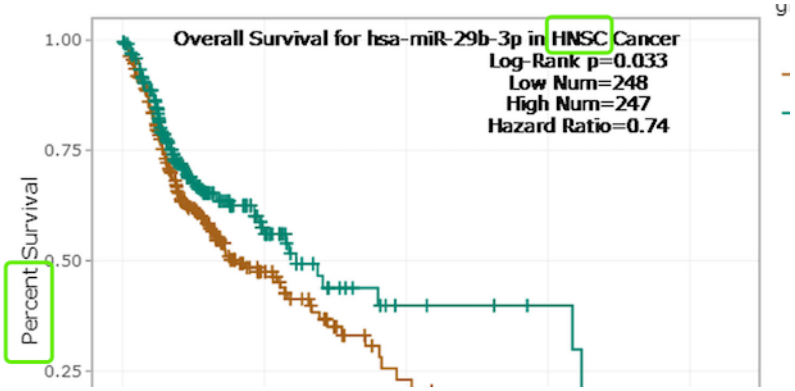
Studies have shown that HNSCC cells may exhibit marked differences in terms of energy metabolism compared with normal cells. Tumor cells absorb more sugar than normal cells. Even under oxygen-rich conditions, tumor cells still preferentially produce lactate. This phenomenon is also known as the Warburg effect or aerobic glycolysis (32). EGFR can activate CCM in tumor cells and plays a key function in aerobic glycolysis. Studies have confirmed that EGFR can directly or indirectly regulate the functional glucose transporter 1 (GLUT1) to maintain glycolysis and the PPPs (33). EGFR regulates glycolysis by regulating the downstream phosphatidylinositol 3 kinase (PI3K)/serine/threonine kinase (Akt)/mammalian target of rapamycin (mTOR) signaling mechanism and promotes the correct localization of GLUT1 to regulate tumor cells adhesion, proliferation, invasion, and migration, which are negatively correlated with prognosis (34). Studies have also indicated that inhibiting the glycolytic pathway can increase the efficacy of targeting EGFR in the treatment of tumors, indicating that glycolysis may be the main oncogenic pathway of EGFR (35).

more tumors than the liver (PFKL) and muscle (PFKM) types. Studies have confirmed that PFKP is overexpressed in tumors, which may indicate poor prognosis (41). When PFKP activity is enhanced, the glycolytic pathway is activated, which promotes DNA biosynthesis and damage repair, thereby stimulating tumor cell growth, migration, and invasion (42). Recent studies have shown that the PFKP-Lactate Dehydrogenase A (LDHA) axis mediates aerobic glycolysis by regulating lactate production in breast cancer cells (43). PFKP inhibition redirects glucose flux to the PPP, largely saving

Reply: Thank you for your reminder. We have revised “studies” to “A study/ a previous study”.

4. Figure 7:

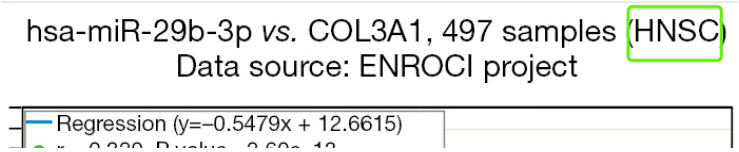
Please delete all “Percent” from your Figure 7 since the rate is 0-1. And please indicate the full name of “HNSC” in the legend.



Reply: We have deleted all “Percent” from your Figure 7

5. Figure 8:

The word should be ““HNSCC”.



Reply: We have changed to “HNSCC”.