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Reviewer A

Comment: Renal cell carcinoma (RCC) is essentially a metabolic disease characterized by a reprogramming of energetic metabolism (PMID: 36960789; PMID: 30983433, PMID: 36430837, PMID: 36310399). In particular the metabolic flux through glycolysis is partitioned (PMID: 29371925, PMID: 28933387, PMID: 25945836), and mitochondrial bioenergetics and OxPhox are impaired, as well as lipid metabolism (PMID: 30538212; PMID: 32861643, PMID: 29371925, PMID: 36430448, PMID: 38540735). In this scenario it has been shown that SNHG3 is an important regulator of cell metabolism and regulates many biological characteristics of renal cancer stem cells (PMID: 37685983). These findings should be referenced and discussed.

In addition, renal cell carcinoma is one of the most immune-infiltrated tumors (PMID: 31527133, PMID: 30738745; PMID: 27063186). Emerging evidence suggests that the activation of specific metabolic pathway have a role in regulating angiogenesis and inflammatory signatures (PMID: 32345771, PMID: 28359744). Features of the tumor microenvironment heavily affect disease biology and may affect responses to systemic therapy (PMID: 38003705; PMID: 37189689; PMID: 33265926; PMID: 36902242; PMID: 37373581). SNHG3 can modulate immune cell infiltration and regulate immunoflogosis. These processes should be explored and better discussed.

Reply: Dear reviewer, thank you for your review of this article and your valuable suggestions. We have carefully read the literature and discussed them in the manuscript. To make reading easier, we append the page and line numbers corresponding to the modified section

Changes in the text: Clear cell renal cell carcinoma (ccRCC) is characterized as a metabolic disease with significant alterations in energy metabolism [26, 27]. There is a distinct partitioning of metabolic flux through glycolysis, as evidenced in recent studies [28, 29, 30]. Additionally, studies have shown that various pathogenic mechanisms such as mitochondrial dysfunction, lipid metabolism, angiogenesis pathogenesis involve in ccRCC [31, 32, 33, 34]. Emerging evidence further indicates that the activation of certain metabolic pathways plays a role in modulating angiogenesis and inflammatory signaling, which can contribute to both the onset and progression of ccRCC and potentially lead to drug resistance [35, 36, 37]. This complexity in pathogenesis presents significant challenges for treatment. (see Page 12, line 305-313)

In recent years, with significant progress achieved in science and technology, more biomarkers, including lncRNAs, have been used for the diagnosis and prognosis of malignancies [38]. (see Page 12, line 315)

Interestingly, SNHG3 can promote the proliferation and metastasis of breast cancer cells by inhibiting mitochondrial oxidative phosphorylation and increasing glycolysis level. This suggests the therapeutic potential of SNHG3 targeting the tumor microenvironment, but whether SNHG3 also has a similar effect in ccRCC remains unclear [45]. Moreover, as ccRCC is one of the most immunologically invasive tumors associated with immune cell infiltration, much literature highlights the potential of immunotherapy, which has garnered significant interest [46, 47, 48, 49]. (see Page 12, line 319-326)

Ghini et al. showed that inhibition of immune checkpoints enhanced immune escape in Non-small cell lung cancers[51]. (see Page 13, line 338-339)

The expression of various molecules can regulate immune activity within the TME of ccRCC [55, 56. 57]. (see Page 13, line 357-358)

In TME, a reduction in T-reg cell numbers leads to an increase in CD8 T cells, helping to slow tumor growth[59]. (see Page 14, line 363-364)

Reviewer B

Comment1: Proper citations are needed for the bioinformatic databases used in the study, such as "TCIA," "TIMER," and others.

Reply1: Thank you very much for your advice. We have attached the corresponding literature after the bioinformatic databases used. (Reference: 17, see Page 4, line95), (Reference: 18, see Page 5, line 99), (Reference: 19, see Page 5, line 101), (Reference: 20, see Page 5, line 104), (Reference: 21, see Page 5, line 106).

Comment2: More details regarding the methods for RNA extraction from ccRCC tissue should be provided, including the amount of tissue used and whether it was fresh tissue or from an FFPE block.

Reply2: We think this is an excellent suggestion. We have made modifications as suggested by the reviewer (see Page 5, line 116-121).

Changes in the test: Clinical samples including ccRCC tissues and adjacent normal tissues (n = 8) were acquired from the same patients who underwent radical

nephrectomy at the First Affiliated Hospital of Guangxi Medical University and rapidly stored in liquid nitrogen for further experiments.

Comment3: The primer sequence for beta-actin and the PCR conditions should be described.

Reply3: We have made modifications as suggested by the reviewer (see Page 6, line 129-134).

Changes in the test: The primer sequences were obtained from Sangon Biotech (Shanghai, China) as follows, SNHG3: Forward 5' - AGTGGTCGCTTCTTCTCCTTG - 3', reverse 5' - GATTGTCAAACCCTCCCTGTTA - 3'. β-actin: Forward 5' - GTCATTCCAAATATGAGATGCGT - 3', reverse 5' - GCTATCACCTCCCCTGTGTG - 3'. Relative expression levels were calculated according to the 2-ΔΔCt method. Statistical analyses were performed with GraphPad Prism.

Comment4: For the siRNA experiments, the sequence and/or catalog numbers of si-SHNG3#1, si-SHNG3#2, and si-NC should be provided. The amount of siRNA used in the experiment should also be described.

Reply3: We have made modifications as suggested by the reviewer (see Page 6, line 134-140).

Changes in the test: The primer sequences were as follows, si-SNHG3-1: Forward 5' - GCUAGGAAUGCACAUUCUUTT - 3', reverse 5' - AAGAAUGUGCAUUCCUAGCTT - 3'. Si-SNHG3-2: Forward 5' - CCUAGCUGAUGAGUUGUAUTT - 3', reverse 5' - AUACAACUCAUCAGCUAGGTT - 3'. Si-NC: Forward 5' - UUCUCCGAACGUGUCACGUTT - 3', reverse 5' - ACGUGACACGUUCGGAGAATT - 3'. A total of 10 pmol of siRNA was transfected into 786-O cells using liposome RNAiMAX (Invitrogen, USA) in each group separately

Comment4: For the wound healing assay, details of the quantitative analysis should be provided, including the number of image fields used, and the software and parameters applied.

Reply4: We have made modifications as suggested by the reviewer (see Page 6, line 150-153).

Changes in the test: We analyzed five image fields for the control group and four image fields for each of the two treatment groups and the blank area was measured by Image J software. Relative migration rate was calculated by normalizing the distance of the blank area at 0h.

Comment5: Scale bars should be included in Fig. 6D.

Reply5: Thank you very much for your advice. The Fig. 6D after adding scale bars have been placed in the tiff file named Fig.6, which is in a compressed package.

Comment6: Details regarding the method of FISH are needed. The size of the nuclei in HK-2 and 786-O cells appears significantly different. Is there any explanation for this observation?

Reply6: We observed a difference in nuclear size between HK-2 and 786-O cells in the FISH experimental images, likely due to the specific field of view selected, which made the nuclear sizes appear different. We checked it carefully and found that in various fields of view under the same magnification, the nuclear sizes of the two cell types were generally similar. However, factors such as the inherent biological characteristics of each cell type and variations in cell cycle stages may contribute to differences in nuclear size. We believe this discrepancy does not affect the interpretation of the experimental results, as our focus is on the localization of SNHG3 rather than the absolute nuclear size. We have ensured that all experimental procedures were consistent across both cell lines to maintain result comparability (see Page 7, line 163-175).

Changes in the test: HK-2 and 786-O cells were cultured on glass slides and fixed with in situ hybridization fixative for 20 minutes at room temperature. Protease K (20 μg/mL) was then applied to the slides for digestion at 37°C. A pre-hybridization solution was added dropwise and incubated at 37°C for 1 hour. After discarding the pre-hybridization solution, a probe-containing hybridization solution was added, and slides were incubated overnight. Following hybridization, slides were washed sequentially with 2× Saline Sodium Citrate (SSC) buffer at 37°C for 10 minutes, 1× SSC at 37°C for 2 intervals of 5 minutes each, and 0.5× SSC at room temperature for 10 minutes. A preheated branch-specific probe hybridization solution (60 μL) was then added dropwise, and the slides were incubated in a humidified chamber at 40°C for 45 minutes. Post-hybridization washes were repeated as above. Nuclei were counterstained with DAPI for 8 minutes. Images were captured under a Nikon upright fluorescence microscope using a 20× objective.

Comment7: The number of replicates for each experiment should be clearly defined.

Reply7: Thanks for your suggestion, we have added the number of replicates for each experiment to the part of method and the text caption of Figure 6 (see Page 7, line 182), (see Page 22, line 621-627).

Changes in the test: Each experimental group at each time point was performed with at least three independent replicates ($n \geq 3$) to ensure the reliability of the results. Each experiment was performed at least three times to ensure reproducibility.

(A) Expression levels of SNHG3 in ccRCC cell lines (n = 6). (B) Expression levels of SNHG3 between normal and tumor samples of ccRCC patients (n = 8). (C) The knockdown efficiency of SNHG3 in 786-O cells was verified by qPCR (n = 5). (D) Wound healing assay was used to detect the effect of SNHG3 on the migration function of tumor cells (n ≥ 5). (E) MTT was used to detect the effect of SNHG3 on the proliferation function of tumor cells (n ≥ 3). (F) FISH detected the distribution of SNHG3 in the nucleus of HK-2 and 786-O cells (n = 3).

Comment8: A comparison between the findings of this study and the previous study by Xu Z, et al., 2021 (doi: 10.21037/tcr-21-1802) should be discussed.

Reply8: Thanks for your suggestion, we have compared and discussed the findings with that study (Xu Z, et al., 2021 (doi: 10.21037/tcr-21-1802)) in the article (see Page 13, line 341-348).

Changes in the test: Our study shares both similarities and distinctions with the work of Xu et al. Both studies demonstrated that SNHG3 regulates the proliferation and metastatic behavior of ccRCC. Xu et al. concentrated on identifying a competing endogenous RNA (ceRNA) regulatory axis, which includes SNHG3, to explore potential therapeutic targets for ccRCC. Our study reveals an association between SNHG3 and immune cell infiltration in ccRCC, suggesting a regulatory link between SNHG3 and the immune microenvironment. This finding highlights the potential of SNHG3 as a therapeutic target for ccRCC from an immunological perspective.

Comment9: The study's limitations and the need for further experimental validation should be addressed.

Reply9: This is a very good suggestion, and we have added a paragraph on the limitations of the study and the need for further experimental verification in the article discussion section as suggested by the reviewer (see Page 15, line 388-397).

Changes in the test: The study still has limitations. Firstly, we did not experimentally illustrate the relevance and possible regulatory role of SNHG3 on immune cell infiltration in ccRCC. By reviewing the literature, we found that most of the results on SNHG3's relationship with immune cell infiltration in the TME of ccRCC or other cancers are largely based on existing database analyses. Second, we did not conduct in vivo experiments to examine the role of SNHG3 in ccRCC regulation. We could refer to the findings of Chong Zhang and colleagues, who reported that knockdown of SNHG3 inhibits ccRCC cell growth and metastasis in vivo [44]. Third, the specific mechanisms by which SNHG3 regulates ccRCC remain unclear, and further research is needed to understand its downstream regulatory network and its effect on immune cells. These limitations should be addressed in future studies.

Comment10: If applicable, the research grant should be provided or acknowledged.

Reply10: We have modified our text as advised (see Page 16, line 407-408).

Changes in the test:

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