Peer Review File

Article Information: https://dx.doi.org/10.21037/tcr-22-503

Reviewer comments

The nature of tumor immune microenvironment (TME) is essential for the head and neck squamous cell carcinomas (HNSCC) initiation, prognosis and response to immunotherapy. In the manuscript "Deregulated YTHDF1 associates with tumor immune microenvironment in head and neck squamous cell carcinomas", authors analyzed the TME infiltration, cancer-immunity cycle activities and YTHDF1-related KEGG pathways.

Couple questions are required to be answered before it will be accepted.

Comment 1: There was a similar report (Nucleic Acids Res. 2020 Apr 17;48(7):3816-3831) about the m6A reader YTHDF1 and cancer in PubMed. What is the novel idea in the paper? Please elaborate clearly in the introduction.

Reply 1: We are very grateful for the Reviewer's comments. Although several similar articles about YTHDF1 and cancer in PubMed, they are mostly on non-squamous cell carcinoma other than HNSCC (1-4). For example, Liu et al. found that YTHDF1 promotes ovarian cancer progression via augmenting EIF3C translation (1). Furthermore, these studies have focused on tumor cells, including proliferation, stemness, metastasis, invasion capability, etc. However, studies on YTHDF1 in TME, especially in the TME of HNSCC, are relatively less, which needs to be further improved.

Compared with other studies, the novelties of our study are mainly as follows:

(1) Among the 24 m6A regulators, we systematically identified YDHDF1 as a potentially critical factor of HNSCC TME. In HNSCC, this is a relatively comprehensive screening, which also gives us and others thinking about the roles of YDHDF1 in HNSCC TME.

(2) Focused on YTHDF1, we firstly reveal the mechanism of high expression of YTHDF1 in HNSCC, which includes genetic factors (copy number amplification), transcriptional regulation mediated by specific transcription factors, and chromatin accessibility.

(3) In contrast to other studies that focused on tumor growth, metastasis, and progression, we are mainly concerned with the TME infiltration, cancer-immunity cycle activities, and YTHDF1-related KEGG pathways in HNSCC.

In summary, we have preliminarily revealed the role of YTHDF1 in HNSCC TME from upstream expression regulation and downstream molecular pathways.

Changes in the text: We have added corresponding statements in the introduction (see Page 4, line 89-92).

Comment 2: The tumor immune microenvironment (TME) is essential for the head and neck squamous cell carcinomas (HNSCC) initiation, prognosis and response to immunotherapy. What were the roles of m6A in TME? Please supplement in the introduction.

Reply 2: We are very grateful for the Reviewer's comments. The complex tumor immune microenvironment (TME) contained stromal cells, distant recruited cells, secreted factors (such as chemokines, cytokines, and growth factors), and new blood vessels. Recently, many studies established a paradigm for understanding m6A mRNA modifications in TME. In the tumor cells, the m6A regulators have crucial roles in tumor cell proliferation, energy metabolism, and immunomodulatory abnormalities (5-7). Specifically, different expressions of m6A regulators associate with variant activities of tumor hallmark-related pathways, which lead to tumorigenesis and tumor progression (5,6). In immune cells, m6A modification is responsible for the series of signal events that result in tumor immunity (8,9). For example, abnormalities of m6A mRNA in dendritic cells powerfully contributed to immune disorders and tumor immune escape (9). Moreover, m6A mRNA methylation also has an impact on extracellular signaling stimulations (including cytokines, chemokines, and high-pressure physical stresses), which affect various aspects of tumor progression (10). In summary, m6A mRNA has been present to be important in TME by precisely regulating tumor and immune cells behavior as well as extracellular signaling.

Changes in the text: We have modified our text as advised (see Page 3-4, line 71-80).

Comment 3: In the title, how to understand the "Deregulated YTHDF1"? It was "downregulated YTHDF1"? Please state clearly.

Reply 3: We thank the Reviewer for carefully evaluating our work. Awfully sorry, this is a mistake that we wrote "Dysregulated" as "Deregulated". As shown in Figure 2, YTHDF1 is upregulated and amplified in HNSCC patients. Based on this, we have changed "Deregulated YTHDF1" to "Upregulated YTHDF1" in the revised manuscript.

Changes in the text: We have modified the title as follows: Upregulated YTHDF1 associates with tumor immune microenvironment in head and neck squamous cell carcinomas.

Comment 4: The YTHDF1 was the crucial topic in the paper. Please make a brief introduction in the part of introduction.

Reply 4: We are very grateful for the Reviewer's suggestion. As suggested, we add a brief introduction to YTHDF1 in the introduction.

Changes in the text: We have modified our text as advised (see Page 4, line 84-88).

Comment 5: Among the 24 m6A regulators, why to focus on YTHDF1? Please state in the results.

Reply 5: Originally, we systematically analyzed the expression of 24 m6A regulators in different groups (including tumor vs. normal, high vs. low Immune score/Stromal score/ ESTIMATE score), which attempt to screen out an m6A regulator that affects TME in HNSCC. As shown in Venn Diagram (Figure 1B), only YTHDF1, ELAVL1, and METTL3 were differentially expressed in each group. Further analysis revealed that YTHDF1 expression was the most significant difference between the two groups based on the median ESTIMATE score (a score that reflects the general condition of TME) and YTHDF1 is the most correlated with the ESTIMATE score. Therefore, we focused on YTHDF1 for further investigations in this study. We have added corresponding statements in the results section.

Changes in the text: We have modified our text as advised (see Page 7-8, line 176-180).

Comment 6: Missing experimental data was the biggest short board in the study. It was advised to validate the associations between YTHDF1 and TME by real world experiments.

Reply 6: We are very grateful for the Reviewer's comments. Indeed, the relationship between

YTHDF1 and HNSCC TME would be better reflected if experimental data were available. However, this paper is a bioinformatics analysis before the subject research. The associations between YTHDF1 and HNSCC TME in real-world experiments will be carried out in the future.

Changes in the text: None

Comment 7: How to apply the YTHDF1 for HNSCC in the future? Please supplement in the discussion.

Reply 7: Studies have shown that YTHDF1 promotes tumor growth in several human cancers, including HNSCC. However, whether YTHDF1 affects the TME of HNSCC remains hitherto unknown. In the future, more studies will focus on the effect of YTHDF1 on the TME of HNSCC, and even may turn to immunotherapy. However, this is a long process, and it takes a lot of basic and clinical research to make this progress. Based on this, more and more experimental new techniques will be referenced in these studies, including single-cell sequencing, multi-omics analysis, and nanomaterial-mediated immunotherapy.

Changes in the text: We have added the relevant content to the discussion (see Page 14, line 344-346).

Reference

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