Peer Review File

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Reviewer Comments

The paper titled "Malignant Tumor Relevance Narrative Review of the PTC-Related LncRNAs" is interesting. This comprehensive analysis not only provides valuable insights into potential regulatory mechanisms of these lncRNAs in PTC but also serves as a reference for exploring their broader regulatory networks within cancer. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1:

1)Please attempt to reveal the biological and clinical relevance of lncRNAs in the occurrence and metastasis of PTC tumors based on the content of this study.

Reply 1:

We would like to express our appreciation for your valuable comments. We suggest that the issue of biological and clinical relevance of lncRNAs in the occurrence and metastasis of papillary thyroid carcinoma (PTC) is extremely valuable. We have provided a more detailed elaboration on this issue. Specifically, the biological and clinical relevance between long non-coding RNAs (LncRNAs) and the occurrence and development of PTC is mainly manifested in the following aspects: 1. LncRNAs can competitively bind to targeted microRNAs (miRNAs that can mediate the degradation of mRNAs), thereby promoting the expression of specific PTC-related mRNAs and proteins. 2. Promote the modification of specific proteins and then affect their protein functions, such as enhancing the activity of metabolism-related enzymes. 3. Stimulate the degradation of tumor suppressor proteins such as P53. 4. Activate specific oncogenic signaling pathways, such as the EPS15L1/EGFR signaling pathway, thereby promoting lymph node metastasis of PTC. All these pathways lead to the occurrence and development of PTC and poor clinical prognosis. However, current studies also show that a

few LncRNAs can enhance the apoptosis of PTC cells and inhibit their proliferation. The mechanism is generally as follows: 1. Competitively bind to targeted miRNAs to promote the expression of specific PTC inhibitory mRNAs and proteins. 2. Inhibit the progression of PTC by regulating tumor-related signaling pathways such as the AKT signaling pathway. Therefore, the role of LncRNAs in the occurrence and development of PTC is very complex and still has a very broad exploration prospect. It is worth noting that the most important mechanism by which LncRNAs affect PTC is through competitively binding to targeted miRNAs, thereby influencing the tumor-related mRNAs and proteins downstream of miRNAs and ultimately affecting the occurrence, development and prognosis of PTC.

Changes in the text:

We have also added the above content about this comment to the Discussion. Please see the revision highlighted in green on page 18, lines 19-29; page 19, lines 1-10.

Comment 2:

2)The description of the methodology in this study is too simplistic. Please add content such as the selected database and the year of literature selection.

Reply 2:

We would like to express our appreciation for your valuable comments. We have added the year of literature selection, the selected databases, and specific information on the search strategy to the main text of the article to facilitate a more in-depth understanding of this review for reviewers and readers.

Changes in the text:

We have also added the above content about this comment to the manuscript. Please see the revision highlighted in green on page 3, lines 23-28; page 4, lines 1-3.

Comment 3:

3)Suggest increasing the impact of LncRNAs on the pathogenesis and potential drug development of PTC.

Reply 3:

Thank you very much for the suggestions you have put forward that can significantly improve the quality of our review. According to the important role of lncRNAs in the pathogenesis of PTC: lncRNAs competitively bind to miRNAs, preventing miRNA-mediated degradation of tumor-related mRNAs and leading to overexpression of tumor-related proteins, exerting a cancer-promoting effect, we can propose corresponding possible treatment plans based on its carcinogenic pathogenesis: 1. Since lncRNAs competitively bind to miRNAs and prevent miRNAs from degrading targeted mRNAs, we can consider developing molecules that mimic miRNA activity to compensate for the role of miRNAs and play an antagonistic role against the carcinogenesis of lncRNAs. Existing studies have shown that this method can be used for the treatment of diseases(1). 2. Since carcinogenic lncRNAs related to PTC are overexpressed in PTC, specific drugs for treating PTC can be developed based on the principle that antisense oligonucleotides (ASOs) and siRNAs specifically bind to the target lncRNAs and then mediate the degradation of the target lncRNAs(2-5). Of course, In this process, there are also some key points that need to be considered: 1. It is necessary to ensure that the drug targets specific target RNA to avoid off-target effects and cause unnecessary side effects; 2. A suitable delivery system needs to be developed to ensure that the drug can be effectively delivered to the target cells and tissues.

Changes in the text:

We have also added the above content about this comment to the manuscript. Please see the revision highlighted in green on page 19, lines 11-21.

Comment 4:

4) Please summarize the current status of lncRNAs in augmenting resistance/sensitivity in

PTC against radiotherapy.

Reply 4:

We are extremely grateful for your valuable suggestions for improving our article. Radiotherapy has currently become an important means of treating patients with PTC(6). However, if patients have radiotherapy resistance, the therapeutic effect will be greatly reduced. Some studies have pointed out that radioiodine resistance is an important cause of death during PTC treatment, and the 10-year survival rate of affected patients is as low as 10%(7). Therefore, studying the influence of lncRNA on the sensitivity or resistance of radiotherapy in PTC patients is a very valuable topic. Existing studies have shown that lncRNA has a complex influence on the radioresistance and radiosensitivity of PTC. Among them, the study by Liang Shi et al. found that LncRNA GLTC can enhance radioiodine resistance in papillary thyroid cancer(8). However, studies by some scholars have found that LncRNA-SLC6A9-5:2 and lncRNA CASC2 can increase the sensitivity of drug-resistant thyroid cancer cells to iodine-131 treatment(9, 10). This clearly indicates that at present, there remain gaps in this field, and there is still extensive research space that merits further exploration.

Changes in the text:

We have also added the above content about this comment to the manuscript. Please see the revision highlighted in green on page 19, lines 22-29; page 20, lines 1-6.

Comment 5:

5) Suggest comparative analysis and screening of lncRNAs significantly correlated with the prognosis of PTC patients, and constructing multi lncRNA features.

Reply 5:

We are very grateful for your highly constructive comments. Screening lncRNAs related to prognosis is of great significance for our future translational applications in clinical practice. Currently, the commonly used lncRNAs related to the prognosis of PTC patients are

determined through statistical analysis and screening through The Cancer Genome Atlas (TCGA) database or other public databases. However, the lncRNAs identified by the above methods have not been verified by in vivo and in vitro experiments, so they do not have high credibility. Therefore, by searching relevant articles, we first determine high-quality PTC-related lncRNAs that have been experimentally verified, and then screen out lncRNAs related to the prognosis of PTC from them, which can effectively ensure their reliability. After careful screening, we found that in PTC patients, lncRNA SOCS2-AS1 and ABHD11-AS1 are significantly associated with poor prognosis(11, 12). High expression of RNF185-AS1 is associated with larger tumor size, lymph node metastasis, and more advanced tumor-node-metastasis stage(13). High expression of lncRNA lnc-MPEG1-1 and MFSD4A-AS1 is significantly associated with lymph node metastasis in PTC patients, also indicating their association with worse prognosis(14, 15). However, lncRNA FOXP4-AS1 is associated with a better prognosis in PTC patients(16). Based on this, we can construct a multi-lncRNA signature (SOCS2-AS1, ABHD11-AS1, RNF185-AS1, lnc-MPEG1-1, MFSD4A-AS1, FOXP4-AS1) to effectively predict the prognosis of PTC patients.

Changes in the text:

We have also added the above content about this comment to the manuscript. Please see the revision highlighted in green on page 20, lines 7-25.

Comment 6:

6) What are the problems and challenges that need to be overcome in the clinical application lncRNA? It is recommended to add relevant content.

Reply 6:

We are extremely grateful for your highly valuable suggestions on our article. At present, there are indeed some issues and challenges that need to be overcome in the clinical application of lncRNAs, which are mainly manifested in the following aspects:1. Specificity issues:

Undesired on-target effects due to uptake in cells other than the target cells, or off-target effects caused by sequence similarities or overdosing to levels much higher than the expected endogenous level. 2. Delivery issues: The "naked", unchemically modified RNA structure is unstable; there is inefficient intracellular delivery of RNA, and endosomal escape mechanisms need to be utilized for improvement; there is a lack of delivery vehicles suitable for targeting specific target organs and cell types. 3. Tolerability issues, such as the recognition of RNA structures by pathogen-associated molecular pattern receptors (such as Toll-like receptors), leading to adverse immune reactions(17, 18). The above challenges also provide valuable directions for future research in this field.

Changes in the text:

We have also added the above content about this comment to the Discussion. Please see the revision highlighted in green on page 21, lines 9-21.

References

1. Gallant-Behm C L,Piper J,Lynch J M, et al. A MicroRNA-29 Mimic (Remlarsen) Represses Extracellular Matrix Expression and Fibroplasia in the Skin. J Invest Dermatol. 2019;139:1073-81.
2. Bennett C F. Therapeutic Antisense Oligonucleotides Are Coming of Age. Annu Rev Med. 2019;70:307-21.
3. Bennett C F, Swayze E E. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annu Rev Pharmacol Toxicol. 2010;50:259-93.
4. Friedrich M, Aigner A. Therapeutic siRNA: State-of-the-Art and Future Perspectives. BioDrugs. 2022;36:549-71.
5. Zhang M M, Bahal R, Rasmussen T P, et al. The growth of siRNA-based therapeutics: Updated clinical studies. Biochem Pharmacol. 2021;189:114432.
6. Haugen B R,Alexander E K,Bible K C, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016;26:1-133.
7. Durante C,Haddy N,Baudin E, et al. Long-term outcome of 444 patients with distant metastases from papillary and follicular thyroid carcinoma: benefits and limits of radioiodine therapy. J Clin Endocrinol Metab. 2006;91:2892-9.
8. Shi L,Duan R,Sun Z, et al. LncRNA GLTC targets LDHA for succinylation and enzymatic activity to promote progression and radioiodine resistance in papillary thyroid cancer. Cell Death Differ. 2023;30:1517-32.
9. Xiang C,Zhang M L,Zhao Q Z, et al. LncRNA-SLC6A9-5:2: A potent sensitizer in 131I-resistant papillary thyroid carcinoma with PARP-1 induction. Oncotarget. 2017;8:22954-67.
10. Tao L, Tian P, Yang L, et al. lncRNA CASC2 Enhances (131)I Sensitivity in Papillary Thyroid Cancer by Sponging miR-155. Biomed Res Int. 2020;2020:7183629.
11. Zhang X,Zhang X,Yang G, et al. LncRNA SOCS2-AS1 promotes the progression of papillary thyroid cancer by destabilizing p53 protein. Biochem Biophys Res Commun. 2023;669:95-102.
12. Wen J,Wang H,Dong T, et al. STAT3-induced upregulation of lncRNA ABHD11-AS1 promotes tumour progression in papillary thyroid carcinoma by regulating miR-1301-3p/STAT3 axis and PI3K/AKT signalling pathway. Cell Prolif. 2019;52:e12569.
13. Liu D, Zhang M, Song Y, et al. RNF185 antisense RNA 1 (RNF185-AS1) promotes proliferation, migration, and invasion in papillary thyroid carcinoma. Anticancer Drugs. 2022;33:595-606.
14. Huang C,Su X,Zhou D L, et al. A diagnostic and predictive lncRNA lnc-MPEG1-1 promotes the proliferation and metastasis of papillary thyroid cancer cells by occupying miR-766-5p. Mol Ther Nucleic Acids. 2022;28:408-22.
15. Liu X,Zhang C,Wang X, et al. Long non-coding RNA MFSD4A-AS1 promotes lymphangiogenesis and lymphatic metastasis of papillary thyroid cancer. Endocr Relat Cancer. 2023;30.
16. Luo X,Gao Q,Zhou T, et al. FOXP4-AS1 Inhibits Papillary Thyroid Carcinoma Proliferation and Migration Through the AKT Signaling Pathway. Front Oncol. 2022;12:900836.
17. Winkle M, El-Daly S M, Fabbri M, et al. Noncoding RNA therapeutics - challenges and potential solutions. Nat Rev Drug Discov. 2021;20:629-51.
18. Nemeth K, Bayraktar R, Ferracin M, et al. Non-coding RNAs in disease: from mechanisms to therapeutics. Nat Rev Genet. 2024;25:211-32.