Dear editorial team and dear reviewers,

I would like to extend our sincere gratitude for the thorough review and constructive feedback provided on our manuscript entitled "**MiRNA: a novel signature in metastasis of esophageal squamous cell carcinoma**" (Manuscript NO.: 90675, Review) . Those comments are valuable and very helpful. We have read through the comments carefully and have made corrections. Revisions in the text are shown in yellow for corrections and additions, and strikethrough font in blue for deletions. The responses to the reviewer's comments are presented below. Based on the instructions provided in your letter, we uploaded the revised manuscript file.

We would like to thank you for allowing us to resubmit a revised copy of the manuscript, and we highly appreciate your time and consideration.

Sincerely,

Responses to the reviewer's comments

Reviewer #1:

The topic approached by the authors of this paper is interesting and overall, I just have some remarks/suggestions to point out. The study provides a comprehensive overview of the importance of miRNAs in metastasis of esophageal squamous cell carcinoma, discussing how miRNAs can regulate gene expression and act as tumor suppressors or oncogenes. It explains how miRNAs can affect the metastasis of ESCC through various genetic mechanisms and by targeting specific and key factors. Specific comments: - The study does not provide a detailed discussion on the mechanisms of miRNA regulation, such as how miRNAs interact with mRNAs or what factors influence their expression. - The study does not provide a comprehensive overview of the potential therapeutic strategies for miRNA-based therapy, such as the development of safe and efficacious delivery systems. - The authors did not discuss the potential side effects of miRNA-based therapies, such as the risk of off-target effects or the potential for miRNA-based therapies to be used in combination with other treatments. - The study does not provide a detailed discussion of the clinical implications of miRNA-based therapies, such as the potential for miRNA-based therapies to improve patient outcomes.

Response: Thank you for giving us the Review comments .

Given the abundant literature that thoroughly examines miRNA regulation, outlines the benefits and obstacles associated with its therapeutic use, and

assesses the current landscape of progress in the field, our work deliberately steered clear of rehashing these known analyses. In response to the insightful review comments received,, we have concentrated our efforts on an in-depth exploration of these subjects within the discussion section of the revised manuscript, leading to their substantial enhancement. The detail is :

CONCLUSION

ESCC is a cancer that originates from the squamous epithelial cells lining the esophagus.It is notorious for its aggressive progression and high propensity for metastasis or dispersion to other regions of the body. In ESCC, metastasis contributes to approximately 90% of cancer-associated fatalities, primarily due to the resulting disruption of digestive function, complications arising from metastasis, an overall deterioration of health, and poor prognostic outcomes. The treatment of metastatic ESCC is intricate, possibly necessitating a combination of therapeutic modalities including surgery, radiation therapy, chemotherapy, and targeted treatments. However, these interventions frequently carry their own array of side effects and complications, and their efficacy can be restricted in the face of metastatic progression. As such, deciphering the mechanisms that underpin metastasis and pioneering effective measures for its prevention and treatment are pivotal objectives in ESCC research.

The metastatic process of ESCC is influenced by a variety of factors, including tumor characteristics, host factors, microenvironmental elements, as well as genetic and epigenetic variations. Growing evidence suggests that miRNA signatures represent an epigenetic mechanism that can profoundly impact various stages of metastasis by regulating gene expression. This regulation occurs through multiple signaling pathways, primarily including the RTK, TGF-β, Wnt/β-catenin, and IL6/Stat3 pathways. Given their regulatory capabilities, miRNAs have surfaced as potential novel diagnostic, prognostic, and therapeutic markers for ESCC metastasis. In their capacity as

diagnostic and prognostic markers, certain miRNAs are consistently overexpressed or underexpressed in ESCC tissues. These miRNAs show negative or positive associations with characteristics of metastasis, such as tumor invasive depth, lymphatic/vascular invasion, lymph node metastasis, and distant metastasis. Intriguingly, specific miRNAs have even been linked to metastatic potential, being associated with the propensity of a tumor to metastasize, the severity of metastasis, and the likely sites of metastatic spread. As therapeutic targets, miRNAs that suppress ESCC metastasis can be restored through the application of miRNA mimics, while those promoting ESCC metastasis can be suppressed using anti-miRNA molecules. The regulation of miRNAs is also influenced by other epigenetic factors, including long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and DNA methylation. Additionally, miRNAs play a crucial role in molding the tumor microenvironment, affecting angiogenesis, and modulating the immune response to tumors. Given their central roles, miRNAs hold significant potential as metastatic biomarkers for both the diagnosis and therapy of ESCC.

Recent advancements in miRNA-based therapy revolve around two principal tactics: the suppression of oncogenic miRNAs to reinstate the activity of their targeted tumor suppressor genes, and the augmentation of tumor suppressor miRNAs to dampen the expression of the oncogenes they regulate. Although they are typically downregulated in cancers, enhancing the expression of tumor suppressor miRNAs can correct the aberrant overexpression of oncogenes they normally control. Additionally, the artificial introduction of tumor suppressor miRNAs can replenish deficient miRNA levels, thereby targeting and disrupting cellular pathways that contribute to tumor development and metastasis. At present, a variety of therapeutic strategies capitalizing on miRNA mimics or inhibitors are being intensively investigated. One such example is MRX34, a liposome-encapsulated miR-34 mimic that has been introduced into clinical trials for the treatment of patients

with primary liver cancer or other malignancies involving the liver. Despite the significant therapeutic potential of miRNAs, several challenges loom must be addressed before their comprehensive integration into clinical practice. One of the foremost challenges in the field of miRNA therapy lies in the potential for off-target effects. These occur when the treatment unintentionally affects genes that have no direct connection to the disease being addressed, leading to unintended and potentially detrimental side effects. Such inadvertent gene regulation can be attributed to the inherent sequence similarities among miRNAs. Moreover, the expansive binding capacity of miRNAs exacerbates this issue; a single miRNA molecule may attach to multiple, disparate mRNAs, thereby simultaneously regulating a host of unrelated genes. This promiscuity in target selection underscores the complexity of achieving precise therapeutic outcomes with miRNA-based interventions. Another substantial challenge in miRNA therapy is achieving effective delivery and ensuring stability. The targeted transport of miRNAs or their inhibitors to designated tissues or cells without loss of function is an ongoing obstacle. Additionally, miRNAs are inherently unstable in the bloodstream due to rapid degradation by nucleases, which calls for the development of sophisticated systems capable of safeguarding these fragile therapeutic agents during delivery. Furthermore, the administration of exogenous miRNA or miRNA mimics may elicit an immune response that could undermine the safety and efficacy of the treatment. Determining the optimal dosage of miRNAs is equally critical, as incorrect dosing could compromise the therapeutic balance, impacting both treatment outcomes and patient well-being. Finally,there are still limitations in accurately predicting miRNA targets, understanding miRNA-mRNA interactions, and quantifying miRNA expression levels, all of which can impede the development of miRNA-based therapies.

As research advances and technology evolves, scientists and healthcare professionals are pioneering methods to navigate the complexities of miRNA

therapy. For instance, the integration of large-scale functional screenings, sophisticated bioinformatics analyses, and strategic chemical modifications are being employed to attenuate off-target effects and bolster specificity. Moreover, an array of innovative delivery mechanisms, including nanocarriers, viral vectors, and exosome-based systems, are being utilized to shield miRNAs from enzymatic degradation and to ensure precise tissue-specific targeting with elevated efficiency. Enhancements in the structural design of miRNAs to assist in evasion of immune detection stand as a testament to the proactive measures being taken to preclude unsolicited immune responses. Additionally, gaining a nuanced understanding of the complex miRNA-mRNA interactions, as well as the epigenetic mechanisms at play, is necessary in order to illuminate new potential applications for miRNA-based interventions.

In summary, persistent explorations in the field of miRNA research are unlocking significant opportunities for improving diagnostic accuracy, refining prognostic predictions, and advancing the therapeutic strategies employed in the battle against cancer. With steadfast commitment to research, the future of miRNAs in medicine appears to be both promising and profound.

Q1:**ABBREVIATIONS**

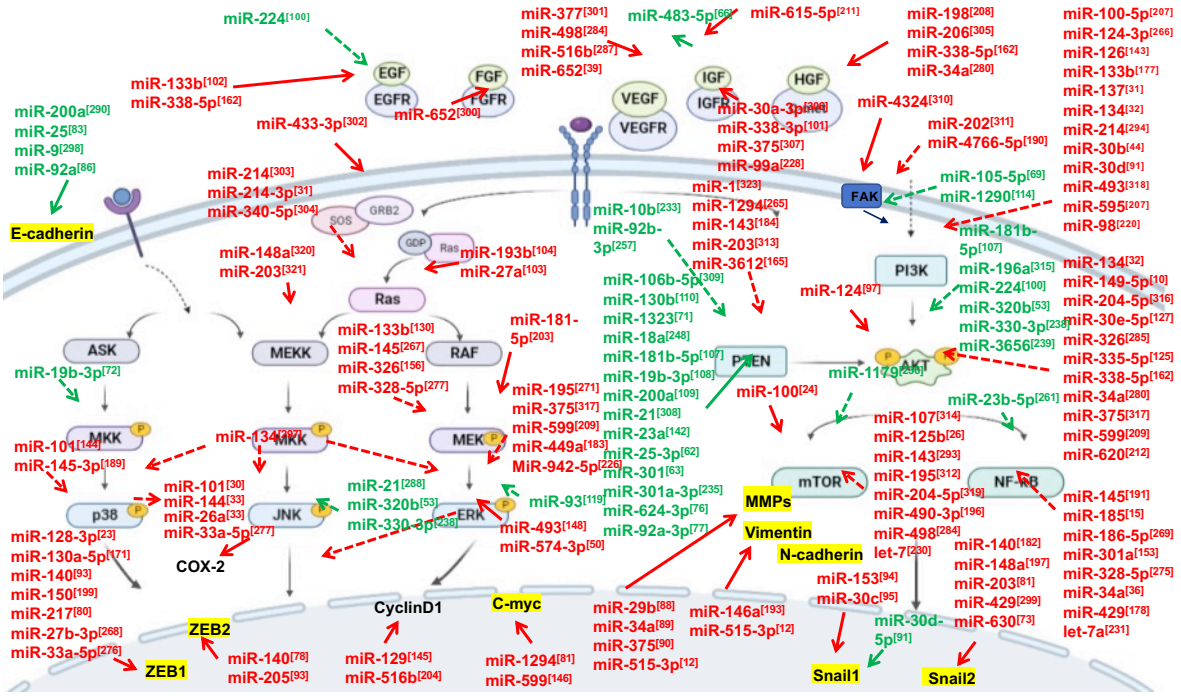
Response:We checked the article to confirm that the abbreviations were defined when they first appeared.

Q2:Table(s) and figure(s),There are 2 Figures and 3 Tables should be improved. All legends are incorrectly formatted and require a general title and explanation for each figure.

Please provide the Figures cited in the original manuscript in the form of PPT. All text can be edited, including A,B, arrows, etc. With respect to the reference

to the Figure, please verify if it is an original image created for the manuscript, if not, please provide the source of the picture and the proof that the Figure has been authorized by the previous publisher or copyright owner to allow it to be redistributed. All legends are incorrectly formatted and require a general title and explanation for each figure. Such as Figure 1 title. A: ; B: ; C: .

Response: We have shown the new tittle in page 117,118.The figure quoted in the original manuscript is provided in PPT form below.



Q3:Please provide all fund documents and the filled conflict-of-interest disclosure form.

Response: We have shown the all fund documents and the filled conflict-of-interest disclosure form in page 3.

Q4: Language evaluation

Language certificate is available in pdf.