Peer Review File

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Reviewer A

The authors present in their manuscript a weighted gene coexpression network analysis (WGCNA) performed on the TCGA data. They claim that they established a reliable model to predict the possibility of distant metastases in differentiated thyroid cancer, which provided an individual risk assessment for clinical decision making. This statement is not true. In my opinion they present a hypothesis, which should be validated on independent clinical material. Validation, which they present, do not concern the correlation between analysed genes with metastases, authors validate only, whether selected genes differ in expression between thyroid cancer and normal cells.

Reply: Thank you for your constructive suggestions. Through comprehensive bioinformatics analysis, this study identified three biomarkers and multiple signaling pathways related to DM in PTCs. More importantly, to confirm the reliability of the three real hub genes, the mRNA expression levels were detected in samples and different thyroid cancer cell lines, which pave the way for subsequent functional experiments. Undeniable, there are several limitations in the current study. The prognostic predictive model is based on TCGA cohort were not validated by experiments and verification with a larger number of samples will be required; however, currently few datasets with complete DM information exist. Also,

our results will require lots of experimental verification.

To our knowledge, this is the first study apply the WGCNA to deeply mine and identify the highly connected hub genes and core modules associated with DM in DTC. and construct an integrated nomogram by combining the hub genes and predictable clinicopathologic factors predict DM, which may have important clinical implications in the improvement of DTC risk stratification and therapeutic decision-making.

Reviewer B

This is an interesting article using a new and useful approach to look for biomarkers able to predict distant metastasis in patients with thyroid cancer. I have some concerns that I would like the authors to discuss:

1. First the language must be carefully revised by a fluent/native English speaker;

Reply: We feel sorry for our poor writings, however, we do invite MJEditor (www.mjeditor.com) help polish our article. And made some changes in the manuscript. These changes will not influence the content and framework of paper. And here we did not list the changes but marked in blue in revised paper. we hope the revised manuscript could be acceptable for you.

2. Authors must use the international consensus to nominate the genes. Genes must be quoted in the text in italic (e.g. TSHR);

Reply: Thanks for your suggestion. We have carefully checked the manuscript and

corrected the errors accordingly.

3. The quantitative REAL-TIME PCR validation is poorly detailed. Authors must describe the correct name of the cell lines, how many times these cell lines were sub cultured (passage), how were these RNAs extracted (which RNA extraction kit was used, which cDNA synthesis kit was used?), which genes were used as endogenous control, and how was the analysis of qPCR conducted? These mRNA values are expressed in fold change?

Reply: Thank you for your constructive suggestions. Four human thyroid cancer cell lines (B-CPAP, K1,TPC-1 and KTC-1) and one human normal thyroid epithelial cell line (Nthy-ori3-1) were used in this study. The cells were cultured in a complete medium composed of 10% fetal bovine serum (Gibco) and 90% RPMI1640 (Gibco, Carlsbad, USA), which was supplemented with 100 mg/mL streptomycin and 100 U/mL penicillin (HyClone). The cells were incubated in 5% CO2 at 37°C and were sub cultured for 10-20 times.

Total RNA was extracted using the AG RNAex Pro RNA extraction kit (AG21101). The RNA was reversely transcribed using PrimeScript™ RT Reagent kit. Reverse transcription was used to compose the first-strand cDNA using 100 ng of total RNA according to the manufacturer's instructions. Subsequently, quantitative real-time polymerase chain reaction (qRT-PCR) analysis was carried out using the TBGreen Premix Ex TaqTMII (Cat # RR047A-5,TaKaRa, Japan). GAPDH was used as internal control. Primer sequences of the related hub genes are listed in Supplementary Table

S1. Relative expression levels were calculated as ratios normalized to GAPDH. The Cq-value for each sample was calculated using the ΔΔCq method, and the results were expressed as 2-ΔΔCq. Real hub genes were identified based on whether the qRT-PCR results exhibited significant differences. The experiments were performed in triplicates.

4. Also, I would like the authors to elaborate on the limitations and on possible implications of mRNA quantification and validation using cell lines.

Reply: Undeniable, there are several limitations about mRNA quantification and validation using cell lines in the current study. First, there are many thyroid cancer cell lines, but we only use 3 of them, resulting in an inevitable selection bias. Second, the expression and function of genetic genes may be changed after sub cultured multiple times. Third, metastatic thyroid cancer tissue samples need further validate. However, we assess the expression of real hub genes for subsequent function experiments.