Peer Review File

Article information: http://dx.doi.org/10.21037/gs-20-589.

Reviewer A

The authors conducted a study to investigate the significance of multi-gene assay of papillary thyroid carcinoma and its implications in the clinical practice. The authors found interesting findings which confirm that BRAF V600E mutation was associated with older age and the aggressiveness of PTC but was independent of lymph node metastasis (LNM). RET/PTC rearrangement suggested more LNM in young patients with PTC. BRAF V600E mutation combined with other gene mutations, termed multi21 gene mutations, could indicate a higher aggressiveness in PTC. Those findings are very interesting and will add more value for the recently available literature given the lack of many evidences in this area of research. Furthermore, the manuscript is well-written and quality of research is scientifically sound.

Response: Many thanks! We greatly appreciate a favorable comment of our manuscript by the reviewer.

Reviewer B

The findings of the paper are interesting but most of which is there in literature. The current version of the paper is fraught by details of the mutational analysis.

Response: We are extremely grateful for your helping suggestion, we apologize for such an ignorance of the detail of the mutational analysis therefore, in the revised version of our manuscript, we added more detailed mutational analysis as the reviewer suggested.

Changes in the text: we add detailed mutational analysis. (page4, line17-22). (page5, line1-12).

Considering the nature of the paper, the authors need to elaborate of the testing kits and gene panel used and its basis as some of the more common mutations have not been analyzed. They can reference to the finding of the TCGA on PTC.

Response: Many thanks for the precious and professional comment! We did a

comprehensive search and reviewed many pieces of literature, which are similar to the Reviewer's recommendations. However, this is our first time to conduct a multi-gene assay, limited to the 57-gene panel, which is provided by the company named USCI (Beijing Youxun Medical Laboratory Co., Ltd.), and it's hard to change a richer gene panel for our current study.

However, based on the suggestion the reviewer provided, we download the most common gene mutation in PTC from TCGA, as shown in Figure 1A. Compared to our research, there are common mutations that have not been analyzed. Interestingly, the prevalence of somatic genetic alterations in PTC was significantly different between our Chinese cohort and TCGA datasets for Americans, as shown in Figure 1B, which was conducted by Chinese scholars. As a result of the small sample size, there are still some common mutations that have not been analyzed, such as TERT, RAS.

Inspired by the reviewer's suggestion, we would launch a multi-gene assay by multiple centers and elaborate on the testing kits and gene panel. The progress of related research will be conducted in near future. Much appreciated for the suggestion, which will be the exact future direction for our further study.

Figure 1A

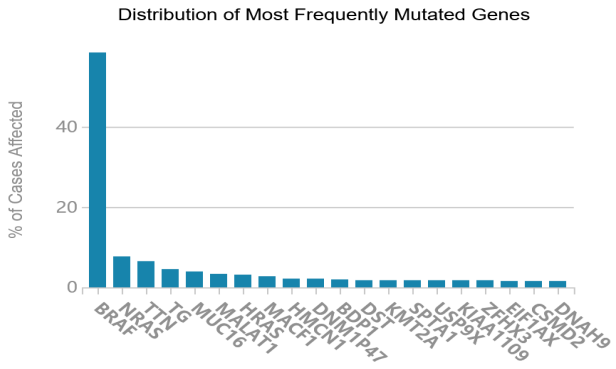
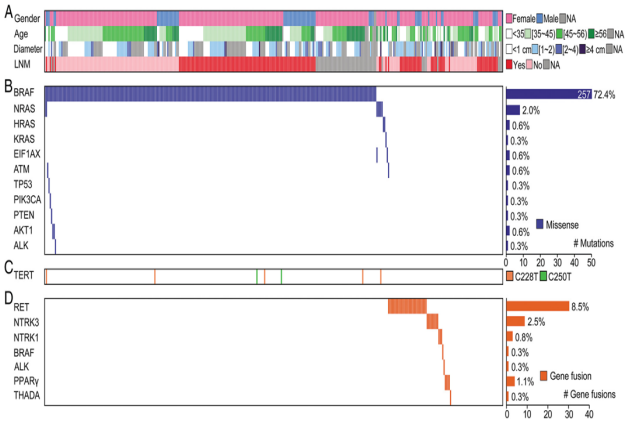


Figure 1B



1. Zhang C, Zheng Y, Li X, Hu X, Qi F, Luo J. Genome-wide mutation profiling and related risk signature for prognosis of papillary renal cell carcinoma. Ann Transl Med. 2019 Sep;7(18):427. doi: 10.21037/atm.2019.08.113. PMID: 31700863; PMCID: PMC6803196.

2.Luo YD, Yu HQ, Liu XY, Huang D, Dai HS, Fang L, Zhang YJ, Lai JJ, Jiang Y, Shuai L, Zhang LD, Chen G, Bie P, Xie CM. Prognostic and predicted significance of Ubqln2 in patients with hepatocellular carcinoma. Cancer Med. 2020 Jun;9(12):4083-4094. doi: 10.1002/cam4.3040. Epub 2020 Apr 15. PMID: 32293796; PMCID: PMC7300399.

3. Poma, A.M., Macerola, E., Torregrossa, L. et al. Using The Cancer Genome Atlas data to refine the 8th edition of the American Joint Committee on Cancer staging for papillary thyroid carcinoma. Endocrine (2020). https://doi.org/10.1007/s12020-020-02434-x

4. Liang J, Cai W, Feng D, et al. Genetic landscape of papillary thyroid carcinoma in the Chinese population. J Pathol 2018;244:215-226.

BRAF mutation is associated with an aggressive disease. The current staging incorporates ageing and not mutation for prognostication. I would like the authors to touch upon this aspect as their findings are in line with the concept touched upon by the short article "BRAF mutation and age in differentiated thyroid cancer risk stratification: Two sides of the same coin. Oral Oncol. 2020 Jul;106:104732. doi: 10.1016/j.oraloncology.2020.104732. Epub 2020 Apr 23. PMID: 32335325."

Response: Many thanks to you for your brilliant comments, we have added contents to

our discussion to make the content richer and more complete.

Changes in the text: we add detailed contents. (page9, line 20-22), (page10, line 1-4) , (page15, line 21-22).

The language needs improvement.

Response: Thank you very much for the Reviewers' comments. We are extremely sorry for the bad writing skills. Considering the Reviewer's suggestion, we have invited a native speaker to proofread and correct our manuscript. These corrections will not influence the content and framework of the paper, which is marked in red in our text.

Changes in the text: (page3, line 10)
(page4, line 8)
(page4, line 10)
(page6, line 6)
(page7, line 12)
(page7, line 14)
(page9, line 15).

The figures can be better, if a larger panel was used a waterfall plot would be better depict the findings.

Response: Much appreciated for the suggestion! We have learned about the waterfall plot but limited to the number of samples and lack of detailed mutational information in our current study, it is difficult to draw the waterfall chart. We have added some limitations in the last paragraph. Therefore, we have launched the multi-gene assay and the database is expanding, the waterfall chart will be used for description and analysis for our further research.

Changes in the text: (page11, line 10-11)

1. Zhang C, Zheng Y, Li X, Hu X, Qi F, Luo J. Genome-wide mutation profiling and related risk signature for prognosis of papillary renal cell carcinoma. Ann Transl

Med. 2019 Sep;7(18):427. doi: 10.21037/atm.2019.08.113. PMID: 31700863; PMCID: PMC6803196.

2.Luo YD, Yu HQ, Liu XY, Huang D, Dai HS, Fang L, Zhang YJ, Lai JJ, Jiang Y, Shuai L, Zhang LD, Chen G, Bie P, Xie CM. Prognostic and predicted significance of Ubqln2 in patients with hepatocellular carcinoma. Cancer Med. 2020 Jun;9(12):4083-4094. doi: 10.1002/cam4.3040. Epub 2020 Apr 15. PMID: 32293796; PMCID: PMC7300399.