Peer Review File

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Title: Late-onset distant metastases confer poor prognosis in patients with well-differentiated thyroid cancer

Reviewer #1

You presented interesting data showing that late-onset distant metastases confer poor prognosis in patients with well-differentiated thyroid cancer (WDTC). It's not a very new concept but used to be in debate. In general, you aimed to investigate the prognostic significance of late-onset of distant metastasis by performing survival analyses and molecular tests in a single-instituted retrospective WDTC cohort. In your 57 WDTC patients with distant metastases, there were 48 cases with papillary thyroid carcinoma (PTC) and 9 cases with follicular thyroid carcinoma (FTC). According the results, the prognosis of WDTC patients was poorer for late metachronously detected metastases than for synchronous or early metachronous metastases. The results were very informative and provided in depth information. However, a few minor points listed below were expected to be replied and enrich the value.

Comment 1: In the "statistical analysis", the authors said "the cancer-specific survival times were calculated from the onset of distant metastasis". However, cause-specific survival is a net survival measure representing survival of a specified cause of death in the absence of other causes of death. Individuals who die of causes other than those specified are considered to be censored. So, I recommend to modify this sentence to be "The cancer-specific survival was defined the survival time in the absence of cancer-related death and calculated from the onset of distant metastasis."
Reply 1: Thank you for the suggestion. We have revised the sentence as suggested by the reviewer.

Comment 2: And the next sentence is "The Kaplan–Meier method was used to plot the disease-specific survival curves." I think the authors mistakenly used "disease-specific survival" but should use "cancer-specific survival", since all their survival analyses were based on cancer-specific survival. Otherwise, they should define "disease-specific survival" and explain this data.
Reply 2: Thank you for pointing out this error. The error has been corrected.

Comment 3: In the results, TERT promoter mutations were associated with radioactive iodine refractivity (P = 0.026) but was not related to cancer-specific survival (P = 0.435), while patients with late-onset (≥5 years) metachronous distant metastases had a higher rate of TERT promoter mutations. It might be discussable. In many published articles, TERT promoter mutations were frequently associated with disease-specific mortality in PTC and FTC. However, in your WDTC cohort with distant metastasis,

the TERT promoter mutations had no significance in cancer-specific survival. We all understand there might be differences in races, institutes, methodology, statistical analyses, and etc. Would the authors provide the discussion to explain why TERT promoter mutations contributed no survival impact in your cohort?

Reply 3: We agree with the reviewer that this was confusing. To address this issue, we have added the following paragraph in the discussion section: "The independent prognostic factors for survival in patients with WDTC include age at diagnosis, tumor-node-metastasis (TNM) staging, and *TERT* promoter mutations (9, 28-30). Previous studies have shown that concurrent *TERT* promoter and *BRAF* or *RAS* mutations had synergistic effects on the worse clinical outcomes of the patients with WDTC (8, 9, 27, 29, 31). In contrast with previous studies, we only enrolled patients who developed distant metastasis. *TERT* promoter mutations were predictive of RAI refractivity but was not related to cancer-specific survival in patients with distant metastasis. Therefore, further studies are needed to evaluate whether *TERT* promoter mutations are predictive of cancer mortality in patients with WDTC and distant metastasis."

Comment 4: In the table 2, why was no available data of radioactive iodine refractivity seen in the multivariate analysis? It was a significant negative predictor in the univariate analysis.

Reply 4: The RAI variable was excluded from the multivariate analysis because the event of interest has not happened to RAI-responsive patients. We have revised the text to improve clarity as follows: "… while no deaths occurred in RAI-responsive patients." We have added the following sentence in the footnote under the table 2: "Cancer-specific death has not happened to RAI-responsive patients"

Comment 5: In the discussion, would the authors kindly provide more discussion in the possible mechanism or hypothesis between late-onset distant metastasis and cancer-specific mortality?

Reply 5: We have added more discussion addressing the effects of older age, *TERT* promoter mutations and thyroglobulin doubling time.

Reviewer #2

This study analyzed 57 well differentiate thyroid carcinomas with distant metastasis and confirmed several essential findings. They were 1) bone metastasis, 2) RAI refractivity, and 3) the onset time of distant metastasis were significantly associated with worse cancer-specific survival. The 5- and 10-year cause-specific survival rates after diagnosis of distant metastasis were 86% and 57%, respectively. Furthermore, this study surprisingly and successfully demonstrated that the late-onset of metachronous distant metastasis was independent predictors for worse cancer-specific survival using multivariate analysis.

Major comments:

Comment 1: Histological confirmation of distant metastasis is a critical factor for the quality of this study because a rare ectopic thyroid tissue in other organs (such as

stromal ovary and mediastinal goiter) mimicked a distant metastasis when elevated serum thyroglobulin was the only evidence for distant metastasis. When this contamination happened in this study, these cases should be found in the synchronous metastasis group and are classified in the non-bone metastasis group. These patients should live long with disease and would result in the same conclusion, such as the late-onset metachronous metastasis, and the bone metastasis had worse cancer-specific survival. Were there any cases in this study whose Ki67 labeling index in the primary thyroid carcinoma was very low (<3%), and histological confirmation of distant metastasis was not available?

Reply 1: Thank you for your invaluable suggestion. We are pleased to say that we could further analyze the thyroglobulin (Tg) doubling-time. We have added a section of Tg doubling time and one more figure, and more discussed the effects of Tg doubling time on distant metastasis and cancer-specific survival. Unfortunately, we could not perform additional study for the Ki67 labeling index due to limited source of tissue samples.

Comment 2: The other possible contamination which reduces the accuracy of this study was the anaplastic transformation or PDC transformation at the metastatic site when the histological confirmation of tumors was not available. It is no doubt that cases with anaplastic change had worse cause-specific survival. Distant metastasis with no detectable RAI uptake (RAI refractoriness) might suggest an anaplastic transformation. You may explain RAI refractoriness as an exchangeable phenomenon with anaplastic and PDC transformation, but it requires an interpretation. Please add a brief statement on how many distant metastases were examined histologically (instead of "confirmed by pathologic examination when available" on page 5), and please deny or confirm anaplastic and PDC transformation in the studied cases.
Reply 2: We initially excluded any case with the component of poorly differentiated or anaplastic thyroid carcinoma in primary or metastatic tumor. For the clarity, we have added the following sentence in the methods section: “We excluded cases showing morphologic evidence for tumoral transformation to poorly differentiated and anaplastic carcinoma (e.g., high mitotic activity, necrosis, solid/trabecular/insular growth pattern, or cellular pleomorphism) in the primary or metastatic tumors.”

Minor comments:
Comment 3: In this study, the cause-specific survival periods after diagnosis of distant metastasis were compared among three groups (synchronous distant metastasis, early-onset metachronous distant metastasis, and late-onset metachronous distant metastasis). Were there any significant differences among the groups when cause-specific survival period after primary thyroid surgery was compared?
Reply 3: We are sorry that the subanalysis was not available due to the small sample size.

Comment 4: Were there any cases whose genetic data were available in both primary thyroid tumor and distant metastasis? Were there any changes between primary tu-

mors and metastatic tumors? If there were, please add the data briefly.

Reply 4: Thank you for your invaluable suggestion. We have added the following paragraph in the results section: "We further analyzed the mutational profiles of matched primary and metastatic tumor samples in six patients. The *BRAF* V600E and *RAS* mutations were found in three and two primary thyroid tumors, respectively, and retained in the matched metastasis. In two cases, the *TERT* promoter mutations were not found in primary thyroid tumors, but were present in their matched metastases. In three cases, the *TERT* promoter mutations were identified in both primary and matched metastatic tumors."

Comment 5: As this focused issue highlights differences and similarities between Asian and Western thyroid practice, this reviewer would like to appreciate if the author adds some comments in this regard.

Reply 5: We have added a paragraph in the discussion section.