Clinical, Pathological, Biochemical and Genomic Characteristics of Poorly Differentiated Thyroid Cancer

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SUMMARY

Poorly differentiated thyroid cancer (PDTC) occupies clinically and histopathologically an intermediate position on a progression spectrum from differentiated thyroid cancer (DTC) to anaplastic thyroid cancer (ATC). However, studies on PDTC have been limited due to relative rarity of the disease and heterogeneous diagnostic criteria. In this work we present a comprehensive study of one of the largest cohort of PDTC patients reported in the literature, diagnosed by criteria of proliferative grading and treated surgically at a tertiary care academic center (Memorial Sloan Kettering Cancer Center; MSKCC), with or without adjuvant therapy during the period of over 24 years. The main objectives of our study were to report on clinicopathological, biochemical and genomic characteristics of PDTC patients, to identify the patterns of treatment failure and to correlate clinicopathological, biochemical and genomic characteristics with outcomes.

PDTC patients tended to present with clinicopathological characteristics that represent adverse prognostic factors in thyroid cancer: older age, higher prevalence of male gender, gross extrathyroidal disease, regional neck metastasis and distant metastasis. Initial surgery resulted in satisfactory 5 year locoregional control rates (81% in PDTC and 56% in PDTC with gross extrathyroid extension (ETE)). Majority of PDTC patients received adjuvant therapy of radioiodine (RAI) and/or external beam radiation therapy (EBRT), however final conclusions on the benefit of adjuvant therapy on disease control are not possible due to small number of patients. We also examined postoperative thyroglobulin (Tg) levels in PDTC patients and found that PDTC patients with undetectable Tg have low risk of recurrence. This finding has to be interpreted in the light of tumor heterogeneity in PDTC and presence of less differentiated component, which warrants close follow-up of PDTC patients. 5 year disease specific survival of PDTC was low (66% in total PDTC and 49% in PDTC with ETE). Indeed, our study showed that PDTC is a highly significant entity, responsible for majority of deaths (57%) from fatal non-anaplastic thyroid cancer. We found that PDTC patients that died of the disease compared to those that survived were: significantly older, presented with larger tumors, local invasion, higher stage and distant metastases. pT4a and M1 stood as significant predictors of worse outcome on multivariate analysis. We identified distant disease as the major cause of death in PDTC patients. Therefore detection of molecular drivers of the disease and development of novel systemic

targeted therapies are neccessary in order to control disease progression and improve overall outcomes.

In order to identify molecular drivers of the disease, we studied genomic profile of PDTC patients. For the first time we report a high frequency of *TERT* promoter mutations in follicular cell-derived thyroid cancer. In addition, overrepresentation of *TERT* promoter mutations in PDTC when compared to PTC may signify a novel biomarker of thyroid cancer progression. *TERT* promoter mutations were significantly associated with *BRAF* or *RAS* mutations in PDTC and ATC, consistent with de novo consensus binding sites in *TERT* promoter for MAPK activated ETS factors. We used ultra deep next-generation sequencing (NGS) technology and MSK-IMPACT targeted assay to profile 341 cancer relevant genes in PDTC. When sequencing of PDTC was compared to the results from sequencing of anaplastic thyroid carcinoma (ATC) and papillary thyroid carcinoma (PTC) of TCGA study, we found genomic instability and accumulation of mutations with thyroid cancer dedifferentiation. This was also accompanied by accumulation of aggressive clinical characteristics and reduced overall survival. Contrary to *BRAF* mutations, *RAS* mutations were more prevalent in advanced tumors in comparison to PTC. *BRAF* mutated PDTC showed significantly higher rates of regional nodal metastases while *RAS* mutated PDTC showed significantly higher rates of distant metastases, consistent with reports on DTC. BRAF-RAS score (BRS), derived from expression patterns of gene panels for BRAFV600E and *RAS* mutated tumors, was preserved in PDTC, i.e. correlated with *BRAF or RAS* mutational status, as shown for PTC. Similarly, we found preservation of thyroid differentiation score (TDS) expression panel in relation to the mutated drivers in PDTC as in PTC. Furthermore, we found the stepwise increase in *TERT* promoter, *TP53* and PI3K pathway effectors mutations between PDTC and ATC. These mutations may therefore represent key genetic events in progression between PDTC and ATC. Copy number alterations (CNA) analysis revealed 1q gain as the most common arm level CNA in PDTC whereby PDTC patients with 1q gains had worse survival rates. In case of PDTC with 22q loss, strong association with *RAS* mutation was present. This can be explained by the transcriptional activation of RAS after the loss of 22q tumor suppressor gene. We also detected mutations of translation initiation factor gene *EIF1AX* in PDTC with comparable frequencies to ATC. *EIF1AX* mutations predicted for worse survival in PDTC patients and may represent a useful marker for risk stratification of PDTC. We also performed ultra deep NGS of PDTC in the context of fatal non-anaplastic

thyroid cancer (FNAT). The most common mutations in FNAT were those of *TERT* promoter. The co-occurrence of *TERT* mutations with *BRAF* or *RAS* mutations in FNAT was consistent with our previous findings in PDTC and ATC. Alterations of MAPK pathway (*BRAF* and *RAS*), *EIF1AX* and *ATM* showed similar prevalence in FNAT compared to ATC, indicating their importance in aggressive thyroid cancer. High incidence of chromosome 1q gain was present in FNAT, consistent with findings of worse survival rates in PDTC with 1q gain. Sequencing of FNAT also revealed presence of novel alterations: fusions *DLG5/RET* and *OSBPL1A/BRAF* and high frequency of *MED12* (14%) and *RBM10* (11%) mutations, suggesting their role in tumor fatality. Comparison between fatal PDTC and nonfatal cases of PDTC revealed that fatal PDTC show a higher frequency of following mutations: *TERT* promoter, *MED12*, *RBM10*, *BRAF*, *HRAS*, *TP53*, *ATM* and *EIF1AX*, highlighting their role in thyroid cancer aggressiveness and progression.

In the present study we report on a comprehensive characterization of PDTC patients, with emphasis on clinicopathological and molecular markers predictive of poor outcome. In addition we report distant disease as the main cause of disease related mortality in PDTC patients. We anticipate that these insights into PDTC biology will contribute to the development of standardized clinical guidelines and development of effective systemic targeted therapies in order to improve the outcomes in patients with PDTC.