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Characterizing parathyroid carcinomas and atypical neoplasms based on the expression of programmed death-ligand 1 expression and the presence of tumor-infiltrating lymphocytes and macrophages ☆

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ABSTRACT

Background: Four distinct tumor microenvironments have been proposed based on the expression of programmed death-ligand 1 and the presence of tumor-infiltrating lymphocytes: immunotype I (adaptive resistance, tumor-infiltrating lymphocytes+ and programmed death-ligand 1+); immunotype II (immunologic ignorance, tumor-infiltrating lymphocytes– and programmed death-ligand 1–); immunotype III (intrinsic induction; tumor-infiltrating lymphocytes– and programmed death-ligand 1+); and immunotype IV (tolerance, tumor-infiltrating lymphocytes+ and programmed death-ligand 1–). These subtypes may predict tumor response to immunotherapy. We hypothesized that parathyroid neoplasms may have tumor immunogenic expression that can later be used to guide treatment.

Methods: We assessed retrospectively the immunohistochemical expression of programmed death-ligand 1 and the presence of tumor-infiltrating lymphocytes (CD3+ and CD8+) and macrophages (CD68+) in parathyroid carcinomas and in atypical parathyroid neoplasms treated at the M. D. Anderson Cancer Center from 1996 to 2016. Using intratumoral digital image analysis, the programmed death-ligand 1 *H* score was calculated with a standardized formula for predominant staining. The tumor-infiltrating lymphocytes per square millimeter of intratumoral areas were quantified.

Results: Within 30 specimens (17 parathyroid carcinomas and 13 atypical parathyroid neoplasms), there was no difference in the median programmed death-ligand 1 *H* score between the two groups ($P = .57$). Four parathyroid carcinoma cases had programmed death-ligand 1 *H* scores ≥ 1 associated with CD3+ and CD8+ tumor cell density; 2 of them had distant metastases. Parathyroid carcinomas had a lesser median CD3+ density ($P = .04$) and a lesser median CD8+ density ($P = .07$) than did atypical parathyroid neoplasms. Median CD68+ density did not differ between groups ($P = .22$).

Conclusion: Parathyroid carcinomas tended to have immune-ignorant and immune-tolerant microenvironments within the neoplasm (immunotypes II and IV). Of the parathyroid carcinoma microenvironments, 17 had patterns of programmed death-ligand 1 and tumor-infiltrating lymphocytes expression (immunotype I), suggesting possible benefit from immunotherapy. In addition, both parathyroid carcinomas and parathyroid neoplasms expressed CD68+. Further exploration of these potential biomarkers as a target in cancer therapies is needed.

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Introduction

Parathyroid neoplasms constitute a heterogeneous group including adenomas, atypical parathyroid neoplasms (ANs), and parathyroid carcinomas (PCs).[1] ANs rarely recur (recurrence-free survival rate = 91%), yet PCs have a high recurrence rate within 5 years after initial parathyroidectomy (recurrence-free survival rate = 60%). For PC patients, effective therapeutic options for multiple recurrences or unresectable metastases are limited, which leads to uncontrolled disease progression[2-4]; the complications of hypercalcemia are usually the final causes of death in these patients. New therapeutic approaches are needed to manage this disease.

Immuno-oncology, in conjunction with the immune targeted therapies, has revolutionized the treatment of many forms of cancer over the past few years. Immune cells express immune checkpoint receptors that, when bound to their ligands, induce an inhibitory signal that downregulates immune response. Each of such tumor microenvironments appears to have an immunogenic signature that can be stratified into 4 distinct groups based on the expression of programmed death-ligand 1 (PD-L1) and the presence of tumor-infiltrating lymphocytes (TILS): immunotype I (adaptive immune resistance, TIL+/PD-L1+); immunotype II (immunologic ignorance, TIL–/PD-L1–); immunotype III (intrinsic induction, TIL–/PD-L1+); and immunotype IV (immune tolerance, TIL+/PD-L1–). These immunogenic subtypes may predict how to approach immunotherapy in each neoplasm on an individual basis, which has been reported to be an important point when selecting combined treatments for cancer.[5-7]

The current literature is lacking with respect to the immune microenvironment in parathyroid neoplasms, and the potential application of immunotherapeutic strategies for PC remains unknown.[5] The aims of this study were to characterize PD-L1 tumor expression and the presence of intratumoral TILs in PCs and ANs and to identify the immune subtypes occurring in these parathyroid neoplasms. We hypothesized that these parathyroid neoplasms may have tumor immunogenic expression, which would be useful for recognizing PCs that are susceptible to immunotherapy.

Methods

From 1996 to 2016, 30 parathyroid neoplasms, including 17 PCs and 13 ANs with archival tissue available from their primary parathyroidectomy, were identified retrospectively from a prospectively collected database at the University of Texas M. D. Anderson Cancer Center. At study conception, all specimens were independently rereviewed by an expert pathologist (MDW), and all diagnoses were confirmed based on absolute World Health Organization diagnostic criteria.[1] For PCs, only absolute criteria of malignancy were considered, including the undisputed presence of any one of the following: invasion of surrounding soft tissues, invasion of surrounding vital structures, vascular invasion, perineural invasion, and histologically documented regional or distant metastases. For ANs, features not fulfilling the absolute criteria of malignancy were noted, such as capsular invasion without extra-parathyroid extension, identifiable mitotic figures, broad intratumoral fibrous bands, coagulative tumor necrosis, diffuse sheet-like monotonous small cells with large nucleus, diffuse cellular atypia, and macronucleoli. Patient clinicopathologic data were retrieved from electronic medical records, and each record was rereviewed meticulously and independently. This study was approved by the institutional review board of the M. D. Anderson Cancer Center.

Consecutive sections of formalin-fixed, paraffin-embedded tumor (5 µm thick) were stained immunohistochemically for anti–PD-L1, -CD3, -CD8, and -CD68 antibodies using an automated staining system (BOND-MAX, Leica Biosystems, Buffalo Grove, IL) according to standard automated and validated protocols. The antibody characteristics and dilutions are summarized in Table 1. Human placental tissue (for PD-L1) and tonsillar tissues (CD3, CD8, and CD68) were used as positive controls.

Expression of PD-L1, CD3, CD8, and CD68 in neoplastic cells was detected using a Novocastra Bond Polymer Refine Detection Kit (Leica Biosystems). Immunostained neoplasm sections were digitally scanned using an Aperio ScanScope Turbo slide scanner (Leica Biosystems) with a 20 × objective magnification and visualized using the ImageScope software program (Leica Biosystems). Digital image analysis was performed using Aperio software, and staining of tumor cells for PD-L1 expression was evaluated using the Aperio Image Toolbox with a pathologist-trained, tumor–membrane-specific algorithm. CD3 and CD8 expression in TILs was analyzed using a quantitative nuclear staining scoring algorithm, and CD68 expression in macrophages was evaluated using a cytoplasmic staining algorithm (Leica Biosystems). Only intratumoral areas for PCs and ANs were selected, and artifacts and stroma were excluded. All aspects of detecting expression and calculation was performed by experts (P.V.) in our institutional molecular pathology laboratory.

Five random 1-mm^2 intratumoral areas were selected for expression analysis of PD-L1, CD3, CD8, and CD68. PD-L1 expression was evaluated for intensity and percentage of neoplasm cells with positive membranous staining. The PD-L1 *H* score for intratumoral areas was based on the sum of the predominant staining intensity levels using the formula $(1 \times [\%\ \text{cells}\ 1+]) + (2 \times [\%\ \text{cells}\ 2+]) + (3 \times [\%\ \text{cells}\ 3+])$. The *H* score ranged from 0 to 300 (maximum value of 300 corresponding to 100% of tumor cells PD-L1 positive). TILS (CD3+ and CD8+) and macrophages were quantified according to the cell density of the neoplasm (number of TILS positive/m^2 of tumor) in the intratumoral compartment. For immune analysis, the selected intratumoral areas were overlaid with sequential immunohistochemical slides to quantify each marker at the same location of each tumor specimen. The data on all neoplasm sections were summarized.

For classification of the PC microenvironment, a PD-L1 H-score ≥1 (1% weak 1+ staining) was used as the cutoff point for PD-L1 positivity.[8] High expression of TILs for this study was defined as specimens with CD3+ and CD8+ TIL density greater than that in the median PC group.[9,10]

Statistical analysis

The Kruskal-Wallis analysis of variance was used to compare the distribution of continuous variables, and the Fisher exact test was used to compare the distribution of categorical variables between the 2 groups. All statistical analyses were performed using R computing language (Version 3.3.1). No statistical adjustment was made for multiple testing.

Results

Table 2 summarizes the demographic, histologic, and clinical characteristics of the PC and AN patients. The PC group had a male predominance and greater median serum parathyroid hormone and calcium levels than the AN group ($P < .05$). Eight of the 17 PC patients (47%) had vascular invasion, and 15 (88%) had soft tissue or muscular invasion. None of the initial operations described capsular rupture or tumor fracture, and none of the local recurrences suggested seeding in the musculature. None of the AN specimens exhibited necrosis or invasion of soft tissues, muscular tissue, or vasculature. All locoregional recurrences ($N = 6$), distant metastases ($N = 5$), and deaths caused by the primary disease ($N = 3$) occurred in the PC group. Of the PC patients who had distant metastases, all of them also had locoregional recurrence, with metastases involving the lungs only in 2 patients and metastases

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