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Composition of the immune microenvironment differs between carcinomas metastatic to the lungs and primary lung carcinomas

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ABSTRACT

Lungs are among the most common sites for development of both primary and metastatic carcinomas. Tumor cells expression (TC) of PD-L1 is an important predictor of the of response to immune check-point inhibition in NSCLC, while the composition of the immune cells (IC) in the tumor microenvironment including PD-L1 + cells is believed to predict responses in tumors of some other primary sites. Total mutational load (TML) and microsatellite instability (MSI) also play a role in response to the immune checkpoint blockade. We investigated immune microenvironment characteristics (PD-1, PD-L1, CD8) of 257 lung biopsies including 81 primary (NSCLC) and 176 metastatic tumors to the lungs. TML and MSI were calculated from massively parallel sequencing (592-gene panel). TC expression of PD-L1 was more common in NSCLC than in metastatic carcinomas (28% vs. 10%, $p = 0.009$), while PD-L1-positive IC were present at relevant percentages (1–5%) exclusively in metastatic carcinomas (31% IC positive vs. 0%, $p < 0.001$). Metastatic carcinomas carried significantly lower TML in comparison with the NSCLCs (6.6 mutations on average vs. 10, $p = 0.01$). All primary NSCLC were microsatellite stable, and only 2 metastatic carcinomas exhibited MSI-H status. The number of PD-1 + and CD8 + tumor infiltrating lymphocytes did not differ significantly between the primary and metastatic carcinomas. Our study revealed significant differences in tumor immune microenvironment (PD-L1 in IC and TC), and its relationship to TML between NSCLC and metastatic cancers. These differences could determine the choice of a predictive biomarker test and subsequently effect(s) of the immune therapy treatments in various advanced cancers.

1. Introduction

Immune checkpoint inhibitors have improved cancer treatment in the recent years, with significant survival benefits in advanced malignancies of diverse lineages (e.g. melanoma, non-small cell lung cancer [NSCLC], renal cell carcinoma, bladder carcinoma, classical Hodgkin lymphoma). Tumor expression of CD274 (programmed cell death 1 ligand 1 or PD-L1) is the most commonly used predictive biomarker for selection of patients for immune check point inhibition, but it is still in need of refinement, particularly differentiating its expression on cancer cells and in the immune cells of the tumor environment [1].

Suppression of the programmed cell death 1 (PD1 encoded by *PDCD1*gene), expressed on activated T-lymphocytes by its ligands PD-L1 and PD-L2 (CD273, *PDCD1LG2*) represent a major immunosuppressive mechanism in the tumor microenvironment [2]. Blockade of that inhibition may reactivate T-cell function and induce their antineoplastic activity [2,3]. PD-L1 expression, measured by immunohistochemistry, can be found in both tumor (cancer) cells (TC) and inflammatory/reactive “immune” cells (IC), and TC of PD-L1has been successfully used to select patients for immune check point inhibitors [4-6]. However, a subset of PD-L1 TC-negative tumors may still respond to the PD-1/PD-L1 blockade while failure to the therapy has been observed in some PD-L1 TC-positive cancers [1]. Therefore, substantial efforts have been invested in refining existing and identifying additional biomarkers that would predict patients' responses to the immune checkpoint inhibition. Consequently, in recurrent and metastatic bladder (urothelial) carcinomas, expression of PD-L1 on immune cells (IC) had been described as a better predictive biomarker to atezolizumab [3,7]. Among other potential predictive biomarkers, increased CD8 + T-cell density and PD-1 overexpression on T-cells, have been investigated [1,2,8-10]. Most recently, an increased expression of the cancer neoantigens and measurement of tumor mutational load and microsatellite instability have emerged as the potent predictors of the response to the immune check point blockade therapies [11,12].

While PD-L1 expression in cancer cells (TC) of the NSCLC has been particularly well characterized, PD-L1 in cancers metastatic to lungs

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(the most common site of dissemination for numerous malignancies) was not. We comparatively analyzed distribution of PD-L1 along with PD-1 and CD8 in neoplastic (TC) and immune cells (IC) of the tumor microenvironment between primary (NSCLC) and metastatic tumors to the lung (carcinomas, sarcomas, melanomas) in order to gain insight in their differences which could lead to improved selection and treatment outcomes for both primary lung carcinomas and for a wide variety of disseminated malignancies.

2. Materials and methods

2.1. Samples

Two-hundred fifty seven formalin-fixed paraffin-embedded tissue samples (81 NSCLC and 176 metastatic tumors to the lung) were profiled at the CLIA/CAP/ISO-certified laboratory (Caris Life Sciences, Phoenix, AZ). Histologic diagnosis for all cases was confirmed by a board certified pathologist (Z.G.) and appropriate slides were used for molecular profiling.

Caris Life Sciences maintains a de-identified database that houses commercial laboratory results stripped of identifiers. The tumor profiling data for this study was obtained from this de-identified database. This analysis was retrospective and only consisted of results that were already stored in the database. This research was compliant with 45 CFR 46.101(b). Therefore, the project was deemed exempt from IRB oversight and consent requirements were waived.

2.2. Immunohistochemistry

The samples were evaluated for PD-L1 (SP142 antibody), PD-1 (NAT105 antibody), and CD8 expression (SP57 antibody) using automated immunohistochemical (IHC) staining methods. Expression of 4 mismatch repair proteins (MMRP) was tested in selected cases (equivocal microsatellite result in NGS analysis) by IHC (MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 antibody; PMS2, EPR3947 antibody).

PD-L1 positivity was defined as expression of membranous staining at ≥ 5% cells in TCs or ICs as suggested earlier [13-16]. Due to the observed low PD-L1 expression in IC (none of the tumors had PD-L1 positivity in ICs exceeding 5%), when IC was statistically analyzed alone we dichotomized PD-L1 IC variable into two categories (< 1% = negative and ≥ 1% = positive).

PD-1 and CD8 expressions were investigated in the IC (T-lymphocytes, histiocytes and dendritic cells) component. Whenever possible, ten consecutive tumor fields were microscopically reviewed under 40 × objective (high-power field, hpf) and the total number of PD-1 + and CD8 + cells was recorded. In case of small biopsies, the whole slides were evaluated for both markers. Mean cohort values for both variables were used for dichotomization in the statistical analysis.

All cases were further stratified into 4 categories based on the presence or absence of PD-L1 expression on TC or ICs (tumor microenvironment, TME, Table 4) [17].

All cases were evaluated by 2 investigators (W.S. and board-certified pathologist Z.G.); discordances in interpretations were resolved at the double headed microscope evaluation.

2.3. Next-generation sequencing (NGS)

Tumor mutational load (TML) was calculated using the massively parallel (next-generation) sequencing (Illumina NextSeq platform). Only missense mutations that were not previously reported as germline variants were used for TML estimation. NGS panel included 592 genes (list of the genes is available here: http://www.carismolecularintelligence.com/solid_tumors_international).

The TML variable was categorized as follows: Low TML (≤ 6); intermediate (7-16) and high TML (≥ 17). This categorization was previously validated, based on the microsatellite instability (MSI) and NGS data comparisons (available here: http://www.carislifesciences.com/platforms/cmi-overview/total-mutational-load-tml/).
Microsatellite instability (MSI) status was determined by sequence analysis of microsatellite repeat tracts in 7317 target loci in the 592-gene panel.

2.4. Statistical methods

The two-tailed Fisher exact test and χ^2 test were applied for the correlation between the variables ($p \leq 0.05$).

3. Results

3.1. Patients and tumor sample characteristics

The study included the samples from 120 male and 137 female patients (mean age: 62.4 for male and 62.6 for female patients; ranges: 12–90 years for male and 7–95 years for female patients).

The histologic subtypes of primary NSCLC included 15 squamous cell carcinomas, 61 adenocarcinomas and 5 other NSCLCs (two adenosquamous, 2 large cell carcinomas and one NSCLC not further specified). Metastatic tumors to the lung, most commonly included carcinomas (n = 126), including colon (n = 51), gynecologic (n = 22), breast (n = 21), head and neck (n = 15), pancreas (n = 10) and kidney (n = 7) primary sites; the remaining 50 metastatic tumors included 15 soft tissue sarcomas, 11 malignant melanomas and 24 cases of miscellaneous histologic types of solid cancers.

Types of specimens submitted for evaluation included 169 small (needle) biopsies (51 NSCLC and 118 metastatic tumors) and 88 surgically resected samples (30 NSCLC and 58 metastatic tumors) (p = 0.57).

3.2. PD-L1 expression in primary (NSCLC) and metastatic tumors to the lung

The results of PD-L1 expression in TC and IC are summarized in Tables 1–3 and illustrative cases of primary NSCLC and metastatic colorectal carcinoma are shown on Figs. 1–2. Specimen type (small vs. surgical biopsy) had no influence on frequency of PD-L1 expression in TCs and ICs (p = 0.23 and 0.86, respectively).

Overall, TC PD-L1 positivity in primary NSCLC was observed in 23 of 81 cases (28%) and in 24 of 176 metastatic tumors (14%) (p = 0.009, Fisher's exact test). Among the 24 positive metastatic tumors, 13 were carcinomas (Table 1). Interestingly, all three PD-L1 + breast carcinomas were triple-negative (ER-/PR-/Her2-) carcinomas while 5 out of six head and neck carcinomas were squamous cell carcinomas. In non-carcinomatous metastases, PD-L1 expression was also observed in 4/11 (36%) metastatic melanomas and 3/12 (20%) soft tissue sarcomas (Table 1).

In adjacent normal lung tissue, PD-L1 expression was observed in alveolar macrophages (positive internal control cell type). However, PD-L1 expression in intratumoral IC was generally low in both cohorts (none of the tumors had IC PD-L1 above 5%).

However, when > 1% IC threshold for positivity was applied, a significantly higher proportion of IC PD-L1staining was observed in metastatic carcinomas than in primary NSCLCs (31% vs. 0%) (p < 0.001) (Tables 1). Notably, other histologic types of metastatic tumors (e.g. melanomas and sarcomas) also showed significantly higher IC PD-L1 expression (13–36% of cases) than NSCLCs. Consequently, tumor immune microenvironment (TME) categories differed significantly between the primary NSCLC and metastatic carcinomas to the lung (Table 3) (see Fig. 3).

No significant difference in TC PD-L1 expression was observed within the two major primary NSCLC subgroups (adenocarcinoma vs. squamous cell carcinoma, p = 0.19, Table 1) whereas significant