Chapter 3

Recurrent genetic alterations of CD274 (PD-L1) and PDCD1GL2 (PD-L2) in T-cell/histiocyte rich large B-cell lymphoma

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Abstract

T-cell/histiocyte-rich large B-cell lymphoma (TCRBCL) is an aggressive subtype of diffuse large B-cell lymphoma (DLBCL) that is characterized histologically by scattered tumor cells within a robust but ineffective inflammatory background. The genetic basis of immune evasion in TCRBCL is currently unknown. We sought to determine whether copy number alterations of CD274 (PD-L1) and PDCD1LG2 (PD-L2) on chromosome 9p24.1, which are defining features of immune escape in classical Hodgkin lymphoma, are also present in TCRBCL. Using a sensitive fluorescent in situ hybridization assay, we found copy number variation or rearrangements of PD-L1 and PD-L2 in 31/33 (93.9%) cases of primary and relapsed TCRBCL, including 8/33 (24.2%) cases with polysomy, 14/33 (42.4%) cases with copy gain, 7/33 (21.2%) cases with amplification, and 2/33 cases (6.1%) with rearrangements. We also found that copy number alterations of 9p24.1 positively correlate with expression of PD-1 ligands. These findings identify a recurrent and targetable mechanism of immune escape in TCRBCL and highlight the need to test the clinical efficacy of PD-1 blockade in this patient population.
Introduction

The large B-cell lymphoma subtype T-cell/histiocyte-rich large B-cell lymphoma (TCRBCL) is characterized by scattered malignant B-cells within an extensive, but ineffective, immune cell infiltrate.\(^1\)\(^-\)\(^3\) Although prior studies have noted frequent tumor PD-L1 expression in TCRBCL\(^4\), the genetic basis of this is unknown. Accordingly, we sought to determine whether copy number gain of CD274 and PDCD1LG2 (hereafter PD-L1 and PD-L2, respectively) on chromosome 9p24.1, a known mechanism of immune escape in classical Hodgkin Lymphoma (cHL)\(^5\),\(^6\), is present in TCRBCL. Using a fluorescent in situ hybridization (FISH) assay, we evaluated copy number alterations and structural rearrangements of PD-L1 and PD-L2 in 33 cases of TCRBCL, and correlated these findings with tumor expression of PD-1 ligands.

Materials and Methods

Case identification

TCRBCL cases were identified through IRB-approved searches of the pathology archives at Brigham and Women’s Hospital, Massachusetts General Hospital, University of Massachusetts Medical School, and the MD Anderson Cancer Center. Thirty-three cases of TCRBCL were successfully evaluated using the FISH and immunohistochemical (IHC) studies described below. Demographic and clinical information were retrieved from the medical record.

Fluorescent in situ hybridization studies

FISH with probes targeting CD274 (PD-L1) and PDCD1LG2 (PD-L2) on 9p24.1 and a control region on chromosome 9 (CEP9) was performed as previously described.\(^6\)-\(^8\) In each case, up to 50 nuclei were analyzed. Nuclei with a target:control probe ratio of three or greater were classified as amplification. Nuclei with a target:control probe ratio of between one and three were classified as copy gain. Nuclei with a target:control probe ratio of one, but with greater than two copies of each probe, were classified as polysomy. Cases were classified into genetic categories according to the highest-level alteration that was observed.

Assessment of PD-1 ligand expression by immunohistochemistry

Double stains for PD-L1/PAX5 and PD-L2/pSTAT3 were performed as previously described.\(^4\),\(^6\),\(^8\) Tumor cells were identified by morphology and PAX5 expression. Fifty tumor cells per case were scored for membranous PD-L1 and PD-L2 expression (0-3+) by two hematopathologists (G.K.G., S.J.R.). A modified H-score for PD-L1 and PD-L2 expression was calculated by multiplying the percentage of positive tumor cells (0-100%) by the average staining intensity (0-3+), which yielded values ranging
from 0 (for 100% of cells with no staining) to 300 (for 100% of cells with 3+ staining).

**Statistics**

All statistical analyses were performed in GraphPad PRISM (Version 7.0, La Jolla).

**Results and Discussion**

The characteristics of the 33 patients included in the study are summarized in Supplemental Table 1. The mean age of patients was 44 years (range 19-71) and there was a male predominance (23/33, 69.7%). Biopsies were predominantly nodal (18/33, 54.5%) with the most frequent extranodal sites including bone marrow, liver, and spleen. The majority of cases were diagnostic biopsies (28/33, 84.8%) with a subset obtained at the time of relapse (5/33, 15.2%).

Using a FISH assay, we found recurrent copy number alterations of PD-L1 and PD-L2 in primary TCRBCL (Figure 1A and 2A). Copy number variation or rearrangement at 9p24.1 was observed in 26/28 cases (92.8%), including 8/28 cases (28.6%) with polysomy, 10/28 cases (35.7%) with copy gain, 6/28 cases (21.4%) with amplification, and 2/28 cases (7.1%) with chromosomal rearrangements. Only 2/28 cases (7.1%) were classified as disomy. In relapsed TCRBCL (Figure 2A, indicated with stars), 4/6 cases (66.7%) had copy gain and 2/6 cases (33.3%) had amplification of 9p24.1. Intra-tumoral heterogeneity in 9p24.1 copy number was observed in the majority of cases of TCRBCL, with the highest observed copy number change frequently accompanied by lower-level changes in subpopulations of tumor cells (Figure 2A). Consistent with the sequence of genetic changes, the percentage of residual (normal) disomic cells was highest in cases with polysomy, intermediate in cases with copy gain and lowest in cases with amplification (Figure 2B). Temporal changes in 9p24.1 copy number were also evident in one patient for whom biopsy material was available from diagnosis and relapse. In this patient (patient 28), the percentage of amplified cells increased from 35% at the time of diagnosis to 100% at the time of relapse one year later after receiving R-CHOP.

Overall, expression of PD-L1 protein was positively associated with the level of genetic alterations (Figure 1 and 2C) and was highest in amplified cases (median PD-L1 H-score 88.9, range 1.6-300), with a subset of cases showing diffuse membranous expression (patients 29, 30, and 31). The percentage of amplified cells relates to the PD-L1 H-score; cases with the lowest percentage of amplified cells (6-18%, patient 25, 26 and 27) had lower PD-L1 H-scores (6.0, 15.4 and 1.6, respectively, Figure 2A and 2C). Moderate levels of PD-L1 expression were typically seen in cases classified as copy gain (median PD-L1 H-score 28.9, range 8-96.2)
and mostly showed positivity in a subset of tumor cells only. Expression of PD-L1 was generally low in cases classified as polysomy (median PD-L1 H-score 17.1, range 1.9-29.6) and disomy (median PD-L1 H-score 4.3, range 4-4.5). In a subset of cases, the level of PD-L1 expression was significantly higher or lower than that predicted by the genetic category, suggesting that additional mechanisms other than 9p24.1 copy number may modify PD-L1 expression in TCRBCL.

Membranous expression of PD-L2 by tumor cells was largely absent, with the exception of one case (patient 33) that showed diffuse membranous expression of PD-L2 (PD-L2 H-score 233.3) in conjunction with a rearrangement of PD-L2 detected in 76% of nuclei by FISH (Figure 1A and 1B). Targeted sequencing of this case was attempted but did not identify a rearrangement partner of PD-L2, possibly due to low tumor cellularity.
Our findings show that genetic alterations of chromosome 9p24.1 are recurrent events in TCRBCL and correlate with expression of PD-1 ligands. The frequency of 9p24.1 copy number gain and PD-1 ligand expression in TCRBCL is high relative to that reported in conventional DLBCL\(^9-11\), although it is less uniform than that observed in cHL.\(^6\) The frequency of polysomy in TCRBCL is slightly higher than in cHL and a lower percentage and absolute 9p24.1 copy number is observed. In addition, there may be differences in co-mutational patterns, EBV infection (absent in TCRBCL), or cell-extrinsic mechanisms.\(^4-6,9,12-14\) Furthermore, although not a primary focus of the present study, we observed that PD-L1 was highly expressed in the tumor microenvironment in TCRBCL, including in cases
that showed only negligible levels of tumor PD-L1 expression. Taken together, these data provide genetic evidence for a likely role of 9p24.1 copy number gain and PD-L1 expression in mediating immune escape in TCRBCL, and highlight an urgent need to define the clinical efficacy of PD-1 blockade in this patient population.

References