Human Pathology (2016) **54**, 17–24

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

In this issue

Evaluation of the diagnostic and prognostic value of PDL1 expression in Hodgkin and B-cell lymphomas☆,☆☆

Thomas Menter MD[a,b], Andrea Bodmer-Haecki MD[a], Stephan Dirnhofer MD[a], Alexandar Tzankov MD[a,*]

[a]*Institute of Pathology, University Hospital Basel, 4031 Basel, Switzerland*
[b]*Department of Histopathology, Hammersmith Hospital Campus, Imperial College Healthcare NHS Trust, W12 0HS London, United Kingdom*

Received 9 December 2015; revised 23 February 2016; accepted 1 March 2016

Keywords:
PDL1;
Hodgkin lymphoma;
B-cell lymphoma;
Immunohistochemistry;
Diagnostic marker

Summary Activation of the programmed death 1 (PD1)/PD1 ligand (PDL1) pathway is important for tumor cells to escape from immune control. The clinical efficacy of therapeutic modulation of the PD1-PDL1 pathway has been recently shown in classical Hodgkin lymphoma (cHL), but little is known about the frequency and diagnostic and prognostic importance of PDL1 expression in lymphomas. The available anti-PDL1 antibody clones E1L3N and SP142 were compared, and a large cohort of Hodgkin lymphomas (n = 280) and B-cell lymphomas (n = 619) was examined for PDL1 using E1L3N. The results were correlated with the expression of other phenotypic markers, interphase fluorescence *in situ* hybridization data of the 9p24.1 region (*PDL1* locus), and the clinical outcome. PDL1 was expressed on more than 5% of tumor cells in 70% of cHL, 54% of nodular lymphocyte-predominant Hodgkin lymphoma, and 35% of primary mediastinal B-cell lymphomas; in the latter, PDL1 expression correlated with *PDL1* gains (ρ = 0.573). PDL1 was expressed in 31% of primary diffuse large B-cell lymphomas (DLBCLs), whereas most other entities did not express PDL1. In cHL, expression of PDL1 correlated with increased numbers of granzyme+ T cells (ρ = 0.251) and CD68+ macrophages (ρ = 0.221) but with decreased numbers of FoxP3+ T cells (ρ = 0.145). In activated B-cell–like DLBCL, PDL1 positively correlated with PD1+ T cells, whereas an inverse correlation with FoxP3+ T cells was seen in the germinal center B-cell–like DLBCL. PDL1 expression can be diagnostically valuable in some gray zones around DLBCL and cHL; it identifies an "immune escape" cluster of cHL and activated B-cell–like DLBCL with increased granzyme+ and PD1+ T cells and macrophages and decreased regulatory T cells.

1. Introduction

The programmed death 1 (PD1)/PD ligand (PDL) pathway is an important checkpoint for the regulation of T-cell–mediated immune responses [1]. It consists of the transmembrane protein PD1/CD279 itself and its 2 ligands PDL1 (B7-H1, CD274) and PDL2 (B7-DC, CD273). These PDLs activate PD1, which

☆ Competing interests: The authors declare to have no competing interests.
☆☆ Funding/Support: Thomas Menter is supported by the Nuovo-Soldati Cancer Research Foundation, Vaduz, Liechtenstein.
* Corresponding author. Institute of Pathology, Schönbeinstrasse 40, 4031 Basel, Switzerland.
E-mail address: alexandar.tzankov@usb.ch (A. Tzankov).

http://dx.doi.org/10.1016/j.humpath.2016.03.005
0046-8177/

results in a reversible inhibition of T-cell activity and proliferation also known as T-cell exhaustion or anergy. This mechanism plays an important physiological role in preventing placenta infiltration by T cells [2] and maintaining self-tolerance [3], which has been verified in animal studies of *pd1* knock-out mice developing several autoimmune diseases [4].

Unfortunately, malignancies also make use of the immunosuppressive effects of the PD1-PDL pathway [5], which is to a part reflected by high levels of PD1-positive tumor infiltrating T-cells. Besides, several tumors are known to express PDL1, being one of the mechanisms of building up a defense line against tumor-infiltrating lymphocytes (TILs) [6]. This applies not only to solid tumors such as lung or breast cancer but also to hematolymphoid neoplasms such as angioimmunoblastic T-cell lymphoma, follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma (PMBCL), and classical Hodgkin lymphoma (cHL), especially the nodular sclerosis subtype [7,8]. Amplifications of the *PDL1* gene locus on 9p24.1 are recurrent in the last 2 entities [9] further underscoring the importance of that pathway. PDL1 expression by immunohistochemistry has been demonstrated in some of the lymphomas listed above only in smaller cohorts or with antibodies shown to more poorly perform so far [10,11]. Because of the fact that intrinsic cancer-specific T cells might be thwarted by PD1-PDL1 interactions [12], targeting PDL1 has become a new approach in tumor therapy [13]. Recently, first very promising results of PD1-PDL1 blockade have been reported in cHL, melanoma, and non–small cell lung cancer [14–16].

So far, the question whether expression of PD1 and PDL1 by immunohistochemistry might also be of diagnostic or prognostic importance in lymphomas has not been answered on larger collectives. It was our aim to investigate the expression of PDL1 at large scale in cHL and various B-cell lymphoma subtypes to draw conclusions both for diagnostic and potential clinicopathological applications.

2. Materials and methods

2.1. Selection and tissue microarray construction

A total number of 899 cases encompassing different lymphoma entities were selected and examined on tissue microarrays (TMAs) and whole slides (Table 1); 15 nodular lymphocyte-predominant Hodgkin lymphomas (NLPHLs), 7 Burkitt lymphomas (BLs), 35 mantle cell lymphomas (MCLs), and 11 T-cell and histiocyte-rich B-cell lymphomas (THRBCLs) were analyzed on whole mount slides. The study was approved by the ethics committee of Northwestern and Central Switzerland (EKNZ 2014-252).

2.2. Immunohistochemistry

To assess the optimal staining for PDL1, 2 different antibodies (clone E1L3N [Cell Signaling, Danvers, MA] and clone SP142 [Roche/Ventana, Rotkreuz, Switzerland]) were evaluated on non–small cell lung cancer tissue as well as reactive lymph node and tonsil tissue (Fig. 1). The staining pattern of both antibodies was as expected: membranous/submembranous with occasional dots, corresponding to the PD1-PDL1 interaction sites (talk given by Dr Banks from Roche/Ventana at the 2015 SH Workshop on Immunodeficiency and Dysregulation at Long Beach, CA, on October 30th); in reactive lymph nodes, the sinus-lining cells as well as macrophages stained positively, as to be assumed. Finally, the E1L3N antibody clone was decided to be used for further evaluations primarily because of the better signal-to-noise results; in addition, this antibody has already been used by other groups showing reliable staining results [17]. Slides were processed on an automated immunostainer (Benchmark, Ventana/Roche, Tucson, AZ). Immunohistochemical staining procedures of all antibodies used in this study are listed in Table 2. This table also contains the staining protocols including the references of staining results obtained in other studies of our group that were used for correlation analyses in the present study.

Only expression of PDL1 on tumor cells was considered in the present study. PDL1 positivity was defined as at least 5% (every 20th) positively staining tumor cells. One-fifth of cases were scored by 2 observers (T. M. and A. T.) to assess reproducibility. To be considered evaluable, cases, especially TMA cases, had to exhibit at least 25% of tissue available for morphologic analysis and at least 1 positively staining tumor-infiltrating macrophage as positive internal control.

Table 1 Staining results of PDL1

Entity	n	+/evaluable (%)
Burkitt lymphoma	13	0/7 (0%)
Chronic lymphocytic leukemia	41	1/37 (3%)
Diffuse large B-cell lymphoma	253	80/260 (31%)
Transformed low-grade lymphomas	17	2 (all former FL)/ 15 (13%)
Follicular lymphoma		
G1-G2	50	3/50 (6%)
G3	10	1/9 (11%)
Lymphoplasmacytic lymphoma	6	0/6 (0%)
Mantle cell lymphoma	35	0/35 (0%)
Marginal zone lymphoma, low grade	73	1/54 (2%)
Marginal zone lymphoma, high grade	59	5/46 (11%)
Primary mediastinal B-cell lymphoma	51	12/33 (36%)
T-cell and histiocyte-rich B-cell lymphoma	11	1/11 (9%)
Nodular sclerosis cHL	152	87/134 (65%)
Mixed cellularity cHL	80	60/74 (81%)
Lymphocyte-rich cHL	14	9/10 (90%)
Lymphocyte-depleted cHL	8	4/6 (67%)
Unclassifiable cHL	11	6/9 (67%)
NLPHL	15	7/13 (54%)