E-Cadherin Expression and Blunted Interferon Response in Blastic Plasmacytoid Dendritic Cell Neoplasm

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Abstract: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is an aggressive neoplasm derived from plasmacytoid dendritic cells (pDCs). In this study, we investigated by immunohistochemical analysis the expression of E-cadherin (EC) on pDCs in reactive lymph nodes and tonsils, bone marrow, and in BPDCN. We compared the expression of EC in BPDCN to that in leukemia cutis (LC) and cutaneous lupus erythematosus (CLE), the latter typically featuring pDC activation. In BPDCN, we also assessed the immunomodulatory activity of malignant pDCs through the expression of several type I interferon (IFN-I) signaling effectors and downstream targets, PD-L1/CD274, and determined the extent of tumor infiltration by CD8-expressing T cells. In reactive lymph nodes and tonsils, pDCs expressed EC, whereas no reactivity was observed in bone marrow pDCs. BPDCN showed EC expression in the malignant pDCs in the vast majority of cutaneous (31/33 cases, 94%), nodal, and spleen localizations (3/3 cases, 100%), whereas it was more variable in the bone marrow (5/13, 38,5%), where tumor cells expressed EC similarly to the skin counterpart in 4 cases and differently in other 4. Notably, EC was undetectable in LC (n = 30) and in juxta-epidermal pDCs in CLE (n = 31). Contrary to CLE showing robust expression of IFN-I-induced proteins MX1 and ISG5 in 20/23 cases (87%), and STAT1 phosphorylation, BPDCN biopsies showed inconsistent levels of these proteins in most cases (85%). Expression of IFN-I-induced genes, *IFI27, IFIT1, ISG15, RSAD2,* and *SIGLEC1,* was also significantly (*P* < 0.05) lower in BPDCN as compared with CLE. In BPDCN, a significantly blunted IFN-I response correlated with a poor CD8+T-cell infiltration and the lack of PD-L1/CD274 expression by the tumor cells. This study identifies EC as a novel pDC marker of diagnostic relevance in BPDCN. The results propose a scenario whereby malignant pDCs through EC-driven signaling promote the blunting of IFN-I signaling and, thereby, the establishment of a poorly immunogenic tumor microenvironment.

Key Words: blastic plasmacytoid dendritic cell neoplasm, E-cadherin, interferon signature

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Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is an aggressive disease, derived from plasmacytoid dendritic cell (pDC) precursors. In BPDCN, skin infiltration by neoplastic cells, followed by leukemic dissemination, represents the most common clinical manifestation of the disease.[1] The diagnosis of BPDCN requires microscopic confirmation of a pDC immunophenotype for the malignant cells using an immunohistochemical panel that ensures a differential diagnosis with other leukemias of myeloid derivation. In particular, expression of CD4 and/or CD56, and 2 or more pDC-specific markers (ie, CD123, CD303/BDCA2, TCL1, E2-2/TCF4), together with negativity for lineage-specific antigens, represent requirements for the diagnosis of BPDCN.[1,2]

At the molecular level, BPDCN is characterized by a complex mutational landscape, including frequent gene losses and recurrent mutations of epigenetic regulators of gene expression, that accumulates during tumor progression and disease dissemination[3] and that may dynamically evolve in different sites of the disease.[4] At present, stem cell transplantation remains the only effective cure for

BPDCN, although accessible to a limited number of patients.[5,6] Recent molecular studies have led to the design of innovative therapeutic approaches[7,8] such as the anti-CD123 immunotoxin, which has been approved for the first-line treatment of BPDCN.[9]

Few studies have evaluated the potential application of immunotherapy in BPDCN. This is partly due to the fact that the immune landscape of this aggressive neoplasm remains poorly characterized, with conflicting data available on expression of the immune checkpoint regulator PD-L1/CD274 in BPDCN cells.[10–12]

Two decades ago, we reported the expression of E-cadherin (EC) in peripheral blood pDCs,[13] and a recent report described the expression of EC on nodal reactive pDCs and on BPDCN neoplastic cells.[14] The role of this molecule in pDCs' functions has been poorly investigated. A recent study showed that EC interferes with type I interferon (IFN-I) production by pDCs. In particular, the homophilic interaction between EC-positive myeloma cells and pDCs was shown to induce TLR9 degradation, followed by suppression of IFN-I production by pDCs.[15]

Cadherin-1 or EC, encoded by the *CDH1* gene, is a transmembrane glycoprotein belonging to the type-1 cadherin family. It is a constituent of adherent junctions that guarantee strong adhesion between neighboring epithelial cells.[16] Beyond adhesion, EC regulates the release of cytokines and chemokines by the epithelium and controls the trans-epithelial passage of immune cells.[17] EC is also expressed by dendritic cells (DCs) and macrophages. In DCs, EC mediates the maturation and migration, and cell polarization through its interaction with the β-catenin complex.[18] These processes are triggered by homophilic EC interactions or heterophilic binding with CD103 (Integrin alpha E) or the inhibitory (natural) killer cell lectin-type receptor G1 (KLRG1).[18] In Langerhans cells, ECs facilitate adhesion to surrounding epithelial cells by homophilic interactions, which prevent their unchecked maturation and migration.[19]

On the basis of these sets of observations, we explored EC expression by normal and neoplastic pDCs. To derive functional correlates, we also analyzed IFN-I-inducible genes in these cells. Finally, based on the established role of IFN-I in triggering immune responses against cancer cells, we analyzed the T-cell content and PD-L1/CD274 expression in BPDCN. Data from this study identify EC as a novel pDC marker of diagnostic relevance in BPDCN. Strikingly, IFN-I production was severely dampened in BPDCN, thus establishing a poorly immunogenic environment.

MATERIALS AND METHODS

Patients and Samples

Cases were selected from the archives of the Pathology Unit of Spedali Civili Brescia and the Department of Dermatology Medical University of Graz (Table 1). Thirteen normal skin (NS) biopsies from consecutive breast reductive surgery were used as negative controls for gene expression

TABLE 1. Cases Included in the Study

	No. Cases
pDC in normal and reactive tissues	
Lymph node	3
Tonsil	2
Bone marrow	6
Cutaneous lupus erythematosus	49
Total	60
Blastic plasmacytoid dendritic cell neoplasm	
Skin	42
Bone marrow	13
Lymph node	2
Spleen	1
Total	58
Leukemia cutis	
Myeloid leukemia, NOS	20
Myeloid leukemia with monocytic differentiation	13
Total	33
Total	151

NOS indicates not otherwise specified.

study. The diagnosis of BPDCN was made according to the updated World Health Organization criteria.[1,20]

The study was carried out in accordance with the Declaration of Helsinki and approved by the local Ethical Committee (protocol number #2900).

Immunohistochemistry

Immunohistochemistry was performed on 4-μm-thick sections of formalin-fixed paraffin-embedded (FFPE) biopsies using primary antibodies as reported in Table Supplemental Digital Content 1 (http://links.lww.com/PAS/B164). Slides were incubated with the primary antibody for 1 hour at room temperature and revealed using the Novolink Polymer Detection System (Leica Biosystems, Milan, Italy), followed by 3'3-diaminobenzidine (Leica Biosystems) as chromogen. Sections were counterstained with hematoxylin.

For double and triple immunohistochemistry, after completing the first immune reaction, the second one was visualized using the Mach 4 Universal AP-Polymer Kit (Biocare Medical, Concord, CA), followed by the Ferangi Blue (Biocare Medical) chromogen; sequentially, the third immune reaction was visualized using the Dako REAL Detection System, Alkaline Phosphatase/RED (Agilent, Santa Clara, CA). EC was stained using BOND-III autostainer (Leica Biosystems) and PD-L1/CD274 using the BenchMark system (Roche Diagnostics).

IFN-I-related proteins MX1, ISG15, and phospho-STAT1 were tested on 13 samples of NS, 24 cutaneous lupus erythematosus (CLE), 24 BPDCN, and 18 LC. Their expression was separately evaluated on epidermal keratinocytes and on the cell infiltrate occurring in the dermis, represented by inflammatory cells in lupus erythematosus and leukemic cells in BPDCN and LC. A semi-quantitative score evaluation was applied, as follows: positive cells ≤5%: score 0; 6% to 25%: score 1; 26% to 50%: score 2; 51% to 75%: score 3; and >75%: score 4.

Digital Image Analysis

Double immunostained slides were digitalized using Aperio ScanScope CS (Leica Biosystems) for quantification purposes. Proprietary algorithms were used in selected areas; count by touch using the ImageScope tool was realized on digital snapshots at ×40 of magnification (snapshot corresponds to 0.157 mm^2).

Quantification of EC+E2-2/TCF4+ pDCs was performed in 3 reactive lymph nodes counting E2-2/TCF4+ pDC clusters and among sparse pDCs (manual count; counting mean for clusters 495.7, mean for sparse cells 261.7).

CD8$^+$Ki67$^+$ T cells in BPDCN were quantified in 9 skin biopsies both in the context of T-cell aggregates as and among sparse intratumoral T cells (manual count; counting mean for aggregates 692.9; mean for intratumoral T cells 108.4) and expressed as CD8$^+$Ki67$^+$ cells/mm^2.

To evaluate PD-L1/CD274 expression in 9 BPDCN skin biopsies, neoplastic tissue was selected using the ImageScope drawing freehand tool to record size area and the IHC nuclear algorithm was run. E2-2/TCF4+ cells were automatically counted, whereas PD-L1/CD274+ cells were counted manually (automatic count is not suitable for stellate/macrophagic cells).

Interferon Signature

Interferon signature was assessed by the quantitative reverse transcription polymerase chain reaction (RT-qPCR) in 40 cases, including 10 samples of NS, 11 CLE, 9 BPDCN, and 10 LC.

RNA extraction was performed by the RNA isolation kit RNeasy FFPE (by Quiagen, Hilden, Germany) according to the manufacturer's protocol. RNA concentration was determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and analyzed by NanoQuant application. RNA was stored at −80°C until use. Reverse transcription to cDNA was performed using the IMPROM-II Reverse Transcriptase Kit (by Promega, Madison, WI). The following gene-specific probes were applied: IFI27 (Hs01086370_m1), IFIT1 (Hs00356631_g1), ISG15 (Hs00192713_m1), RSAD2 (Hs01057264_m1), SIGLEC1 (Hs00988063_m1), and MX1 (Hs00895598_m1). PCR was performed with the TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) and expression study was performed using the ABI PRISMTM 7000 Sequence Detection System (Applied Biosystems).

The relative abundance of primary transcript was normalized on the expression levels of the 18S mitochondrial RNA subunit (Hs999999001_s1). For each probe, the individual data were compared with a single calibrator (ie, NS5) with gene expression values close to the average of the control group. Relative quantification was calculated as 2 −ΔΔCt, that is, normalizing the quantification variation with respect to control data. The median fold change of *IFI27*, *IFIT1*, *ISG15*, *RSAD2*, and *SIGLEC1* was used to create an interferon score for each case, which was compared with the median of previously collected healthy controls (twice the SD of the mean of the healthy skin group), as previously reported.[21] Expression values of *MX1* were excluded from interferon score calculation because the corresponding protein is normally expressed on pDCs.[22,23]

Statistical Analysis

Statistical analyses used in the study were Fisher test for protein evaluation and 1-way analysis of variance for gene expression, after logarithmic transformation. Comparison of statistics between the samples was performed using the Mann-Whitney test for 2-sided nonparametric values. The sample size was calculated relative to the analysis of gene expression and assuming a normal distribution of gene expression data, after logarithmic transformation, and hypothesizing a 1-way analysis of variance model balanced with 4 groups. Assuming a significance level of 5%, a minimum power of 80%, and an effect size of $f = 0.56$ (according to Cohen definition), we obtain a total sample size of at least 40 patients.

RESULTS

EC is Expressed in Terminally Differentiated pDCs

EC expression in pDCs was initially determined in lymphoid tissues of healthy individuals. To this end, FFPE sections of the bone marrow, tonsils, and reactive lymph nodes were stained with antibodies specific to EC and the pDC marker E2-2/TCF4 alone, or in combination with anti-CD303/BDCA2. In the bone marrow, where erythroid precursors regularly express EC (Figure Supplemental Digital Content 3, http://links.lww.com/PAS/B165), scattered pDCs were negative for EC expression (Figs. 1A, B), whereas in reactive lymph nodes and tonsils, the majority of pDCs were positive, with no differences between cells organized in clusters or those dispersed in the extra-follicular area ($P = 0.2$) (Figs. 1C, D).

We subsequently tested EC expression in pDCs occurring in skin biopsies of CLE (n = 31), a disease typically associated with pDCs recruitment and IFN-I activation and release.[24–27] Double stain for EC and E2-2/TCF4 revealed a significant difference in EC expression in pDCs depending on their localization. Specifically, whereas in dermal aggregates most pDCs expressed EC (Figs. 2A, C), no immunoreactivity was observed in the cells located at the dermal-epithelial junction (Figs. 2A, B, D, E) in areas of severe epithelial damage.[24]

EC Retention in BPDCN

Forty-nine cases of BPDCN were stained for EC, and 39 of these (80%) were positive (Table 2, Table Supplemental Digital Content 2, http://links.lww.com/PAS/B166). In the majority of the cases, EC immunoreactivity was strong on the cell membrane, combined in some instances with a granular cytoplasmic staining (Figs. 3A–E). Notably, in 31 of 33 (94%) BPDCN cases localized to the skin (Table 2, Figs. 3A, B) and in all nodal and splenic cases (3/3, Figs. 3C, D), EC expression was homogeneously detected in the neoplastic cells.

The 2 cases of cutaneous BPDCN that were negative for EC showed the classic BPDCN phenotype, 1 with expression of 5 specific markers (ie, CD4, CD56, TCL1, CD123, BDCA2) and the second lacking CD123 (Table Supplemental Digital Content 2, http://links.lww.com/PAS/B166).
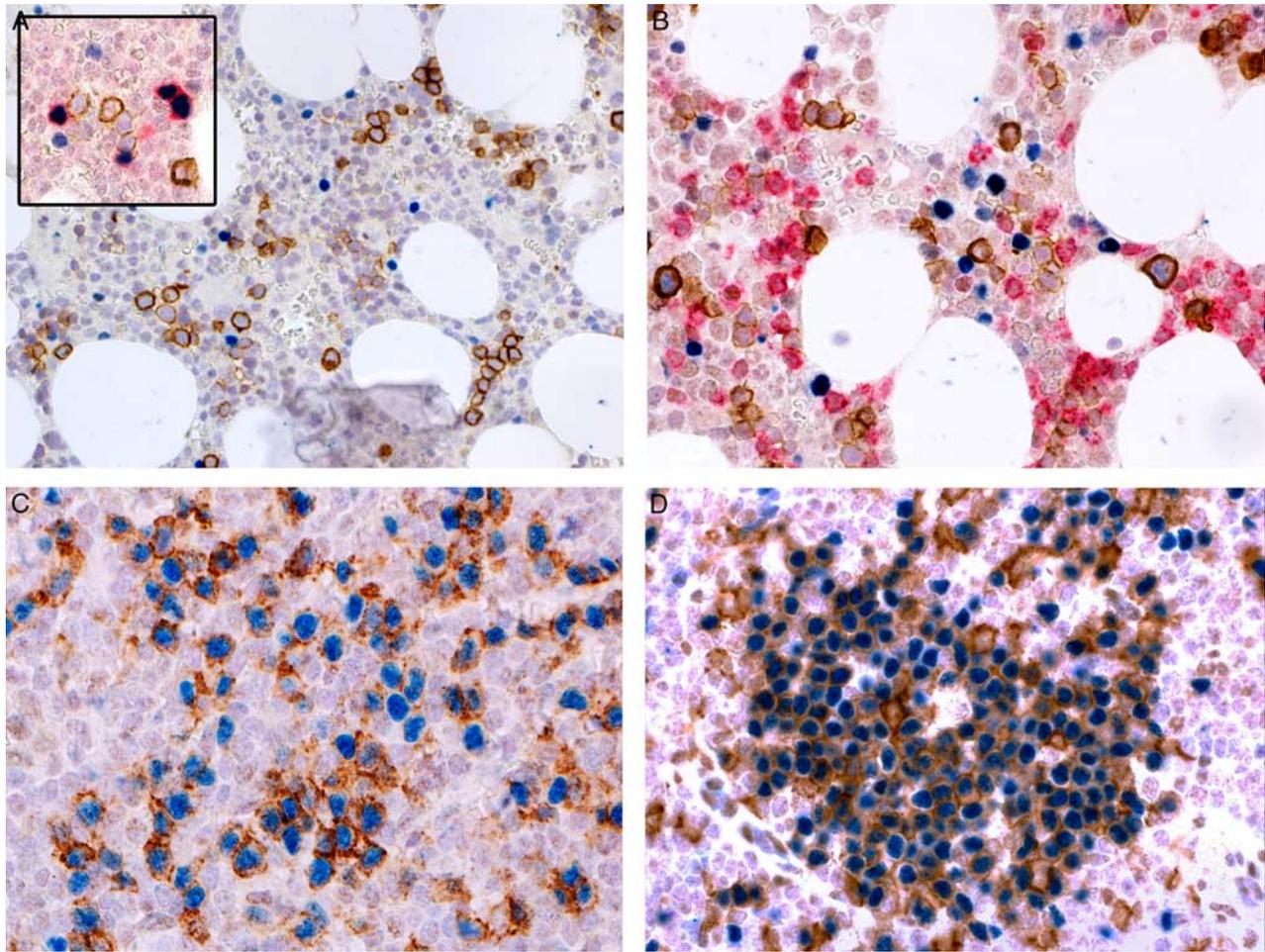
FIGURE 1. E-cadherin expression on pDCs in normal bone marrow and reactive lymph node. A and B, Normal bone marrow containing scattered pDCs identified by E2-2/TCF4 (blue) (A, B), or E2-2/TCF4 (blue) and CD303 (red) (A, inset). pDCs are negative for E-cadherin (brown), which is strongly positive on the cell membrane of erythroid precursors (A, B). B, The E2-2/TCF4-positive pDCs are also negative for CD2 (red). C and D, In a reactive lymph node, E2-2/TCF4-positive (blue) pDCs occurring in the interfollicular area express E-cadherin (brown). Other E-cadherin-positive E2-2/TCF4-negative cells are represented by macrophages.

Remarkably, EC expression was more variable in BPDCN involving the bone marrow, with only 5/13 cases showing positive staining (38%, Table 2, Figs. 3E, F and Table Supplemental Digital Content 2, http://links.lww.com/PAS/B166). By comparing skin and the corresponding bone marrow biopsy (n=8), we found concordant reactivity of EC in 4 of 8 (50%) BPDCN cases (3 positive and 1 negative for EC), whereas the remaining 4 cases were consistently EC positive in the skin, but failed to show immunoreactivity in the bone marrow. In contrast to BPDCN, all samples of leukemia cutis (LC, n=30) were negative for EC expression (Table 2, Figs. 3G, H). Notably, the latter included 4 cases with the partial BPDCN phenotype, including positivity for CD4, CD56, and CD123 in 1 case, and CD4 and CD56 in 3 cases.

Defective IFN-I Production in BPDCN

Expression of EC in pDCs has been linked to impaired IFN-I responses by these cells.[15] Therefore, we tested the expression of a representative set of IFN-I-induced factors in BPDCN and compared it with NS and CLE. At the protein level, we tested by immunohistochemistry the expression of MX1 and ISG15, and evaluated the activation status of the JAK-STAT pathway measuring STAT1 Y701 phosphorylation (pSTAT1).

Whereas in NS, MX1, ISG15, and pSTAT1 were undetectable, in CLE, the 3 factors were regularly (MX1: 22/24 cases, 92%; ISG15: 20/23 cases 87%, pSTAT1: 20/23 cases, 87%) and strongly expressed (score: 3 to 4 for each of the 3 markers; Table 3 and Figures, Supplemental Digital Content 1, http://links.lww.com/PAS/B167 and 2, http://links.lww.com/PAS/B168) by both dermal and epidermal cellular components, in agreement with chronic local activation of IFN-I responses.[28] In comparison, epidermal immunoreactivity for MX1, ISG15, and pSTAT-1 was significantly (P < 0.0001) under-represented in BPDCN cases (MX1: 6/22 cases, 27%; ISG15: 2/14 cases, 14%; pSTAT1: 4/21 cases, 5%) (Table 3, Figure, Supplemental Digital Content 1, http://links.lww.com/PAS/B167), pointing to an interference with the local activation of IFN-I responses.
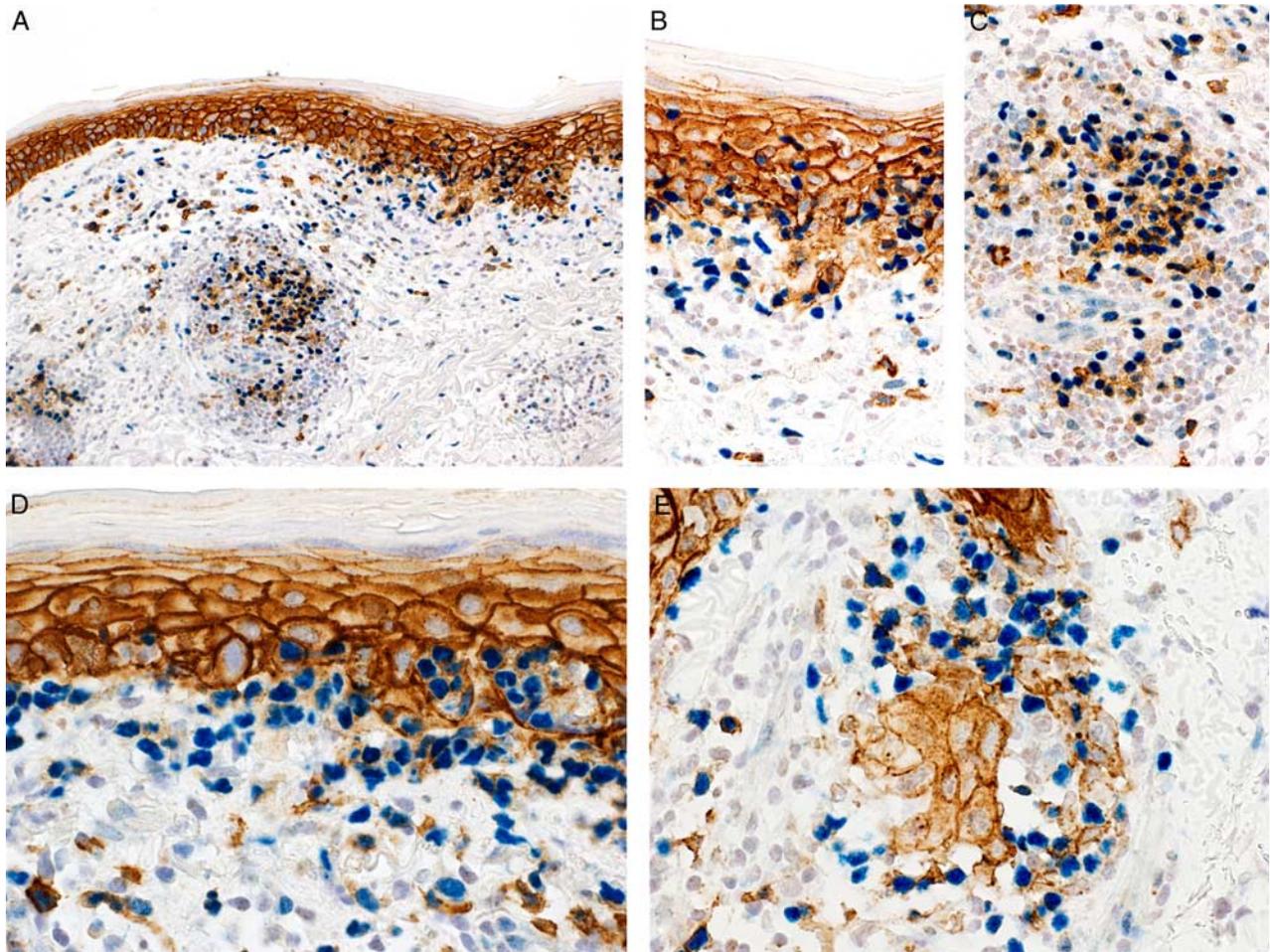
FIGURE 2. E-cadherin expression on pDCs in cutaneous lupus eythematosus. A–E, In 2 distinct cases of cutaneous lupus ery-thematosus (A–E), the pDCs identified by E2-2/TCF4 (blue) occurring at the dermo-epidermal junction (A, B, D) or close to remnants of a hair follicle epithelium (E) do not express E-cadherin, whereas they are positive in a dermal cluster (A, C). E-cadherin is strongly positive in the epithelium.

In agreement with previous reports describing MX1 expression in reactive[13,23] and neoplastic[20,29] pDCs, 19 of 24 cases of BPDCN cases showed robust (score 3 to 4) expression of the MX1 protein in tumor cells (79%; Fig-ures, Supplemental Digital Content 1, http://links.lww. com/PAS/B167 and 2, http://links.lww.com/PAS/B168),

TABLE 2. Summary of EC-expression on Reactive and Neoplastic pDCs in Different Samples

Diagnosis			Samples	E-cadherin Expression	
				Positive	**Negative**
pDCs in reactive tissues and inflammatory conditions	42	3	Lymph nodes	3	0
		2	Tonsil	2	0
		6	Bone marrow	0	6
		31	Cutaneous lupus erythematosus	0	31*
Blastic plasmacytoid dendritic cell neoplasm	49	33	Skin	31	2
		13	Bone marrow (5)†	5 (2)	8 (3)
		2	Lymph node	2	0
		1	Spleen	1	0
Leukemia cutis	30	19	AML	0	19
		11	AMoL	0	11

*pDCs at the dermal-epidermal junction.
†Between brackets are the number of bone marrow biopsies without other sites analyzed for EC.
AML indicates acute myeloid leukemia; AMoL, acute myeloid leukemia with monocytic differentiation.
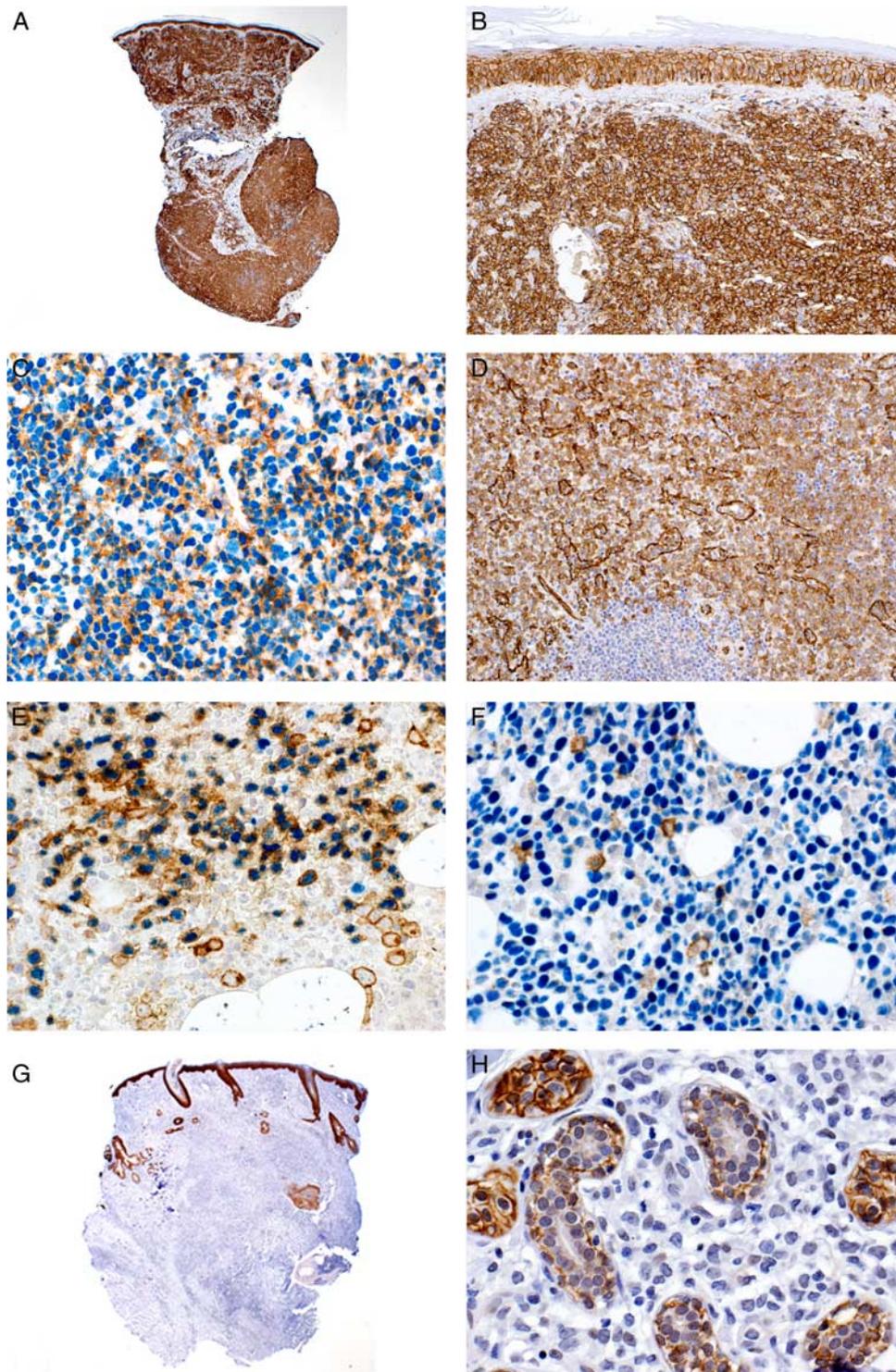
FIGURE 3. E-cadherin expression in blastic plasmacytoid dendritic cell neoplasm and leukemia cutis. A–F, Cases of BPDCN involving the skin (A, B), lymph node (C), spleen (D), and bone marrow (E, F). Tumor cells strongly express E-cadherin (brown) in the skin and spleen; positivity is more variable in the lymph node (double stain for E2-2/TCF4 [blue] and E-cadherin [brown]). E and F, show 2 cases of BPDCN involving the bone marrow, where tumor cells are, respectively, positive and negative for E-cadherin. In both biopsies, note positive erythroid precursors. G and H, In leukemia cutis, leukemic cells diffusely involving the dermis (G) including the peri-eccrine tissue (H) are negative for E-cadherin.

TABLE 3. Scores of Interferon-related Protein Expression (MX1, ISG15, and pSTAT1) on Keratinocytes and in the Dermal Inflammatory or Neoplastic Infiltrate

	MX1		ISG15		p-STAT1	
Score	Inflamm Infiltrate	Keratinocytes	Inflamm Infiltrate	Keratinocytes	Inflamm Infiltrate	Keratinocytes
NS						
0	NA	11	NA	13	NA	12
1	NA	2	NA	0	NA	1
2	NA	0	NA	0	NA	0
3	NA	0	NA	0	NA	0
4	NA	0	NA	0	NA	0
Total		13		13		13
CLE						
0	1	1	1	1	1	2
1	0	1	1	1	2	1
2	1	0	3	1	5	1
3	1	0	5	3	8	7
4	21	22	13	17	7	12
Total	24	24	23	23	23	23

	MX1		ISG15		p-STAT1	
	Neoplasia	Keratinocytes	Neoplasia	Keratinocytes	Neoplasia	Keratinocytes
BPDCN						
0	0	10	1	7	8	12
1	5	4	3	4	6	2
2	0	2	0	1	5	3
3	3	2	4	1	2	3
4	16	4	8	1	2	1
Total	24	22	16	14	23	21
LC						
0	10	12	7	12	9	10
1	3	2	5	2	6	2
2	1	0	1	1	3	2
3	3	1	1	0	0	1
4	1	2	1	0	0	2
Total	18	17	15	15	18	17

NA indicates not applicable.

whereas a variable expression pattern was found for ISG15 (12/16 cases, 75%) and pSTAT1 (4/23 cases, 17%) (Figures, Supplemental Digital Content 1 http://links.lww.com/PAS/B167 and 2, http://links.lww.com/PAS/B168). Together, these results point to a dysregulation of IFN-I signaling by the malignant pDCs of BPDCN. To determine the status of the IFN-I-controlled transcriptional response in BPDCN, we measured transcripts for the IFN-I stimulated genes *IFI27*, *IFIT1*, *ISG15*, *MX1*, *RSAD2*, and *SIGLEC1*.

In keeping with the results obtained at the protein level, the IFN-I gene expression signature was strongly induced in CLE when compared with NS (Fig. 4A, Table, Supplemental Digital Content 3, http://links.lww.com/PAS/B169). Notably, transcript levels for all except *SIGLEC1* genes were significantly ($P < 0.05$) lower in BPDCN as compared with CLE. The differences were confirmed by calculating the Interferon scores[21] measured in NS, CLE, and BPDCN (Fig. 4B, Table, Supplemental Digital Content 3, http://links.lww.com/PAS/B169) ($P < 0.01$).

Defective T-Cell Contexture and Lack of PD-L1/CD274 Expression in BPDCN

Our data point to a defective IFN-I response in BPDCN, at variance with CLE lesions, the latter representing active inflammatory lesions sustained by the action of T-effector cells.[24,30,31] Production of IFN-I,[32] together with the extent of recruitment and activation of innate and/or adaptive immune cells at sites of neoplastic growth, dictates the outcome of anti-cancer immune responses.[33–38]

To start exploring the immune contexture of BPDCN, we analyzed the distribution and proliferation status of $CD8^+T$ cells. $CD8^+T$ cells were found in perivascular aggregates at the invasive margin and, more rarely, intermingled with tumor cells. $CD8^+T$-cell density in the center of the tumor, as measured by absolute cell counts, was low ($CD8^+cells/mm^2$: from 1.06 to 31.68, mean 15.44, median 18.86; Fig. 5E) compared with other human cancers[39]; moreover, only a minority of $CD8^+$ T cells were actively proliferating, as determined by the expression of the Ki67 marker (intratumoral infiltrate: mean: 11.6%; median: 20.53%; perivascular aggregates: mean: 20.46%; median: 21.09%; not shown). These values are significantly low when compared with immunogenic tumors.

Finally, we determined the expression pattern of the immune checkpoint regulator PD-L1/CD274 in a representative set of BPDCN cases (n = 9). Immunoreactivity for PD-L1/CD274 was detected in all BPDCN cases (range: 5.46 to 64.42 cells/mm², mean: 24.32/mm², median: 13.05/mm²) (Fig. 5A). On the basis of morphology, expressions of CD68,
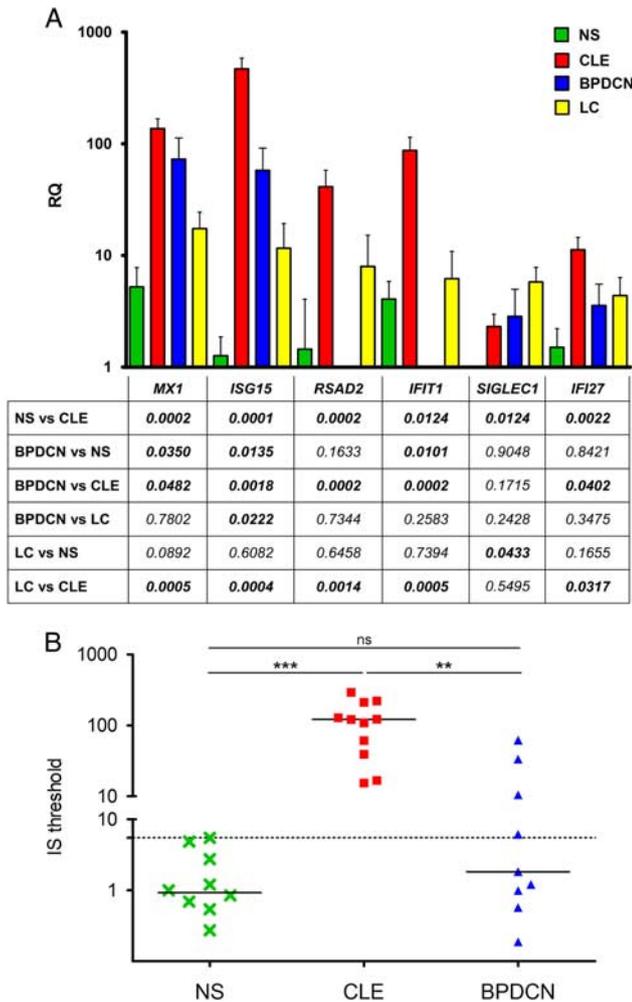
FIGURE 4. Interferon signature in normal skin, cutaneous lupus erythematosus, blastic plasmacytoid dendritic cell neoplasm, and leukemia cutis. A, The histogram and table show the expression values (RQ) on a logarithmic scale of IFN-I-stimulated genes and corresponding *P*-values of gene expression comparison among groups (significant *P*-values are in bold). B, Scattered plot of interferon scores (IS) for each case and group median values (horizontal lines) of normal skin (NS), cutaneous lupus erythematosus (CLE), and blastic plasmacytoid dendritic cell neoplasm (BPDCN). The IS thresold, calculated as previously reported[21] on the NS group, is equal to 5.4946. **$P < 0.01$; ***$P < 0.001$; ns, *P*-value not significant.

MAFB,[40–42] and E2-2/TCF4, PD-L1/CD274-positive cells were mainly represented by E2-2/TCF4-negative, CD68, and MAFB double-positive macrophages (Figs. 5C, D). In our cohort, E2-2/TCF4^{+} BPDCN neoplastic cells lacked reactivity for PD-L1/CD274 even in tumor areas enriched by CD3^{+} T lymphocytes (Fig. 5B).

DISCUSSION

Two main findings relevant to BPDCN emerged from this study. We provide evidence for EC as a novel BPDCN diagnostic marker and establish the molecular basis for a blunted IFN-I response in this disease with

implications in anti-tumor immunity. We surmise a functional connection between these 2 observations, although future investigations are required to obtain experimental proof for this hypothesis.

EC was originally identified as a marker of circulating pDCs by Cella et al.[13] This study substantiates the expression of EC in pDCs in peripheral lymphoid tissues and its absence on those residing in the bone marrow. As pDC maturation completes in the bone marrow, driven by the action of the transcription factor E2-2/TCF4,[43] our findings suggest that EC is induced upon migration of the cells to secondary lymphoid organs and/or to extra lymphoid tissues.

The lack of EC in pDCs residing at the epidermal-dermal junction in CLE biopsies highlights the dynamic nature of this regulation possibly influenced by the state of activation of the cells linked to IFN-I production and by the microenvironment, rich in CD8^{+} cytotoxic T cells.[24–27,30]

Extending recent data,[14] our work identified robust and consistent EC expression in BPDCN tumor cells of a cohort of 49 cases from different body sites. In particular, EC expression was observed in 31 of 33 cases (94%) of cutaneous BPDCN, identifying EC as a novel diagnostic marker for this disease, to be combined with established positive and negative stains.[20,29] In particular, EC emerged from our study to be useful in the differential diagnosis of BPDCN with LC, particularly in cases of AML, which co-express 2 or more BPDCN-associated antigens, such as CD4, CD56, and CD123.[44,45] An additional non-negligible factor is that, in contrast to some BPDCN markers (ie, TCL1, CD303/BDCA2, and E2-2/TCF4), EC is widely used and available in the majority of pathology laboratories.

Because of the possibility for comparison in a representative set of cases, EC expression in malignant pDCs in the skin and bone marrow of the same patient revealed discordance in 50% of the cases, with EC positivity consistently found in the skin while absent in the bone marrow. This discrepancy is unlikely due to preanalytical variables (ie, loss of antigen immune reactivity due to bone decalcification procedures) since all biopsies contained positive controls represented by erythroid precursors[46] (Figure, Supplemental Digital Content 3, http://links.lww.com/PAS/B165). We cannot exclude that the bone marrow subset of neoplastic pDCs in BPDCN may preferentially consist of less differentiated cells, or that microenvironmental factors contribute to the preferential silencing of EC in the bone marrow subset of tumoral pDCs.

EC in immune cells influences the maturation and migration of DC and macrophages,[18] whereas its downregulation is associated with a tolerogenic phenotype.[47] EC is expressed by alternatively activated macrophages (M2) and is involved in the formation of multinucleated giant cells and osteoclasts.[18] In Langerhans cells, EC confers homophilic adhesion to keratinocytes and clustering. Conversely, EC inhibition induces Langerhans cell cluster disaggregation, maturation, and loss of dendritic morphology.[19] In these cells, the EC signal is triggered by
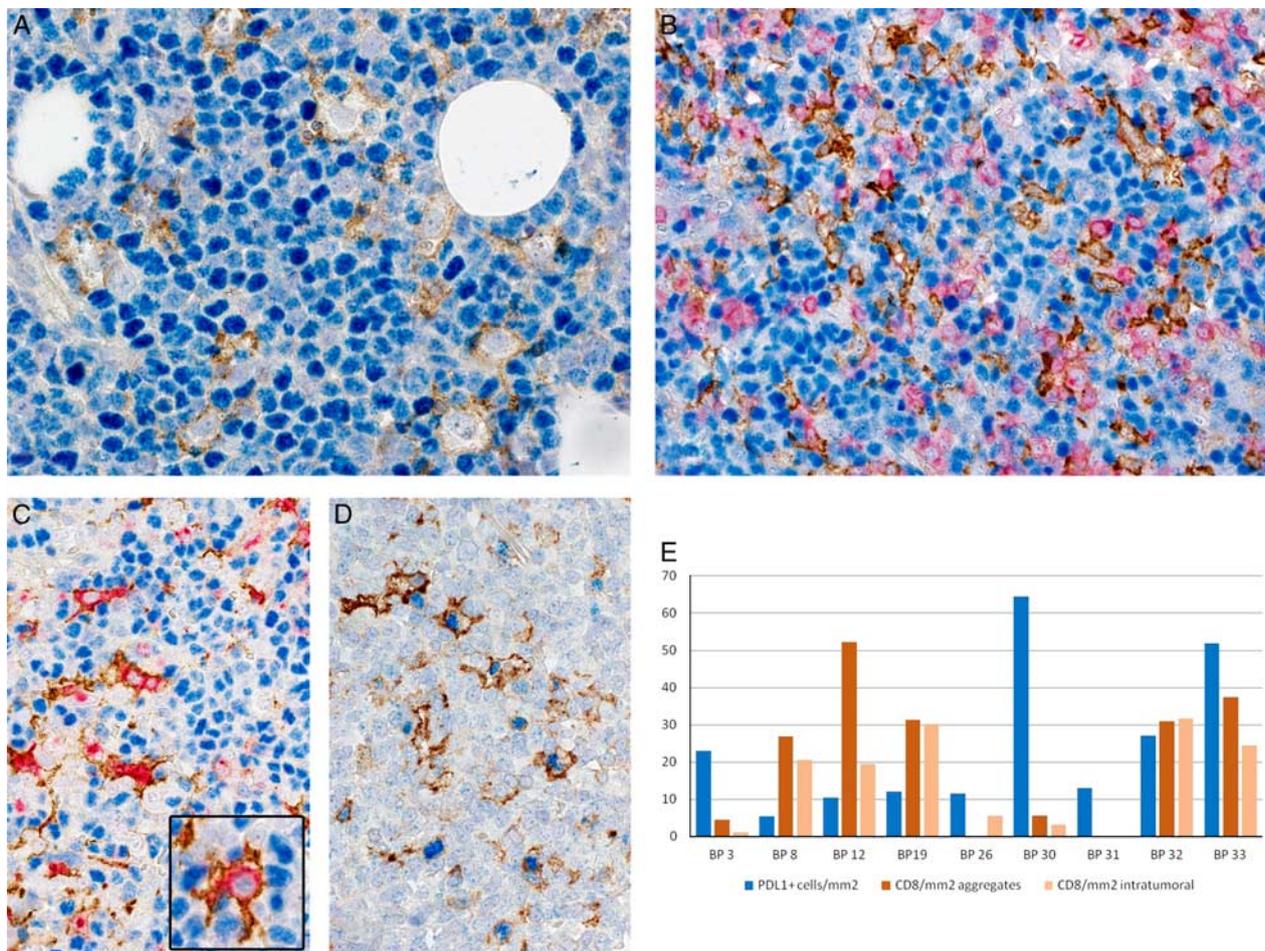
FIGURE 5. PD-L1/CD274 expression and CD8^{+} T-cell microenvironment in BPDCN. A and B, Double stain for E2-2/TCF4 (blue) and PD-L1/CD274 (brown) shows that BPDCN tumor cells are negative for PD-L1 (A), whereas scattered macrophages are positive. In (B), anti-CD3 has been added (red), showing BPDCN cells are PD-L1/CD274^{−} also in areas rich in T lymphocytes; note the numerous dendritic-shaped PD-L1-positive cells. The PD-L1/CD274-positive cells (brown) are largely represented by macrophages, co-expressing CD68/PGM1 (red, in C including the inset, at higher magnification) or MAFB (blue, D). In (C and inset), E2-2/TCF4 (blue)-positive BPDCN cells are also shown. E, The histogram shows the number of PD-L1/CD274-positive cells (blue bar) and CD8^{+} T-lymphocytes as peritumoral aggregates (brown) and as intratumoral scattered cells (light brown) in 9 cases of BPDCN.

homophilic or heterophilic (with CD103 or KLRG1) interactions.[18]

EC regulation and function in pDCs are poorly explored. It has been shown that homophilic interactions between EC-positive myeloma cells and pDCs induce degradation of TLR9 and consequent suppression of IFN-I production by pDCs.[15] We speculate that EC-mediated homophilic interactions in pDC clusters and BPDCN lesions may result in regulation of downstream signals.

Data on the IFN-I production by BPDCN are highly controversial, with reports showing no or low IFN-I in culture supernatants of tumor cells stimulated with TLR9 ligands[48–50] and others demonstrating IFN-I activity in long-term pDCs tumor cell culture.[51,52] Moreover, heterogeneity in IFN-I release has been related to the site of origin of tumor cells or to molecular alterations acquired during clonal expansion.[53,54] Notably, patients with BPDCN lack systemic lupus erythematosus-like symptoms or clinical features asso-ciated with high IFN-I production[20,55,56] or treatment,[57] suggesting normal tissue and serum levels of this cytokine.

The data obtained in the present study indicate that in BPDCN, IFN-I signaling is defective. Gene expression studies of BPDCN[58,59] also failed to detect expression of IFN-I-related genes, in keeping with the closer relationship of BPDCN to "resting" rather than "activated" pDCs. Renosi et al[60] hypothesized that the frequent deletions of the 9p21.3 region encoding IFN genes occurring in BPDCN are responsible for the lack of IFN-I production. Alternatively, these authors proposed that a fraction of BPDCN might derive from the recently described pDC-like DCs (also known as AS-DC), a subset of DC that express CD123 and CD303, in addition to CD2 and AXL, and typically lack IFN-I production.[61,62] However, AS-DC are mostly negative for the transcription factor E2-2/TCF4, which is expressed by the majority of BPDCN, as shown in this and in previous studies.[2,59]

In line with the lack of IFN-I production and consequent defective local immune response, quantification within BPDCN of the CD8$^+$ T-cell contexture revealed a desert immune environment. Indeed, the density of infiltrating CD8$^+$ T-lymphocytes in BPDCN was significantly lower when compared with solid tumors that have been found to be immunotherapy-responsive.[37,38,63,64] In this respect, we found that PD-L1/CD274 reactivity in tissues involved in BPDCN was limited to macrophages, sparing the neoplastic cell compartment, in line with a recent study.[11]

In conclusion, this study identifies EC as a novel pDC marker of diagnostic value in BPDCN. The results propose a scenario where malignant pDCs, through EC expression and signaling, are responsible of blunting IFN-I signaling, and, thereby, of the establishment of a poorly immunogenic tumor microenvironment.

The evidence of a substantial T-cell-exclusion identifies BPDCN as a "cold" tumor. In this setting, new therapeutic strategies could be investigated in BPDCN, with the aim of exploiting the cytotoxic power of T cells in a TCR-independent manner and to divert T cells to form a cytolytic synapse (eg, bispecific T-cell engagers or CAR T-cells).[65]

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