Biomarkers, Genomics, Proteomics, and Gene Regulation

CD137 Is Expressed in Follicular Dendritic Cell Tumors and in Classical Hodgkin and T-Cell Lymphomas

Diagnostic and Therapeutic Implications

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CD137 (also known as 4-1BB and TNFRSF9) is a member of the tumor necrosis factor receptor superfamily. Originally identified as a costimulatory molecule expressed by activated T cells and NK cells, CD137 is also expressed by follicular dendritic cells, monocytes, mast cells, granulocytes, and endothelial cells. Anti-CD137 immunotherapy has recently shown promise as a treatment for solid tumors and lymphoid malignancies in preclinical models. We defined the expression of CD137 protein in both normal and neoplastic hematolymphoid tissue. CD137 protein is expressed by follicular dendritic cells in the germinal center and scattered paracortical T cells, but not by normal germinal-center B cells, bone marrow progenitor cells, or maturing thymocytes. CD137 protein is expressed by a select group of hematolymphoid tumors, including classical Hodgkin lymphoma, T-cell and NK/T-cell lymphomas, and follicular dendritic cells neoplasms. CD137 is a novel diagnostic marker of these tumors and suggests a possible target for tumor-directed antibody therapy. *(Am J Pathol 2012, 181:795–803; http://dx.doi.org/10.1016/j.ajpath.2012.05.015)*

CD137 (also known as 4-1BB and TNFRSF9) is a transmembrane glycoprotein of the tumor necrosis factor receptor superfamily. CD137 is broadly expressed by cells of the human immune system, including activated CD8^{+} and CD4^{+} T cells, activated natural killer (NK) cells, follicular dendritic cells (FDCs), monocytes, and a minor subpopulation of activated B cells within the germinal center (GC).[1–7] CD137 can also be expressed by mesenchymal cells, including endothelial cells, chondrocytes, and cells of the central nervous system.[8–10] Although CD137 has diverse roles in the immune response, one key function is to promote the survival of both T cells and dendritic cells by binding the cognate ligand CD137L (4-1BBL).[11] In T cells and FDCs, engagement of CD137 by CD137L leads to activation of the NF-κB pathway and up-regulation of antiapoptotic members of the Bcl-2 family including Bfl-1 and Bcl-X$_L$.[12,13] In addition to prosurvival signals, CD137 expression by FDCs has been shown to promote T-cell-dependent humoral immune responses by stimulating the proliferation of GC T and B cells.[7,14]

Given the important role of CD137 in providing costimulatory and prosurvival signals, the use of anti-CD137 antibodies as a therapeutic strategy for both solid-organ and hematolymphoid malignancies has been the subject of intense investigation.[15] Preclinical data using agonistic blocking anti-CD137 antibodies has shown promising antitumor efficacy in mouse models of sarcoma, mastocytoma, and plasma cell myeloma.[16,17] More recently, members of our research group demonstrated that agonistic antibodies to CD137 could also eradicate estab-

lished B-cell lymphomas in a murine model.[18] The anti-tumor effect of the agonistic anti-CD137 antibody was shown to be mediated by NK cells and CD8^{+} T cells, because the lymphoma cells did not express CD137. Also, in tumor samples that included both tumor cells and infiltrating immune cells, the expression of the mRNA encoding CD137 (*TNFRSF9*) and a marker of germinal-center B cells (*LMO2*) predicted outcome in patients with diffuse large B-cell lymphoma (DLBCL).[19] The prognostic effect of CD137 was contributed by the tumor microenvironment and likely correlates with the frequency of an activated T-cell subset, because no correlation was seen between CD137 expression and total T-cell infiltration within tumors. Although the precise mechanism of the antitumor effect of CD137 immunomodulation remains unclear, there is evidence to suggest that CD137 expression by dendritic cells and intratumoral blood vessels may also play a role in the antitumor effect of CD137.[20–23]

In contrast to the well-characterized expression of CD137 by normal immune-cell subsets, the expression of CD137 by neoplastic hematolymphoid cells is poorly understood. CD137 expression is inducible in human T-cell leukemia cell lines *in vitro*,[1,24] but few data have been available on the expression pattern of CD137 in primary human hematolymphoid tumors. In the present study, we characterized CD137 expression in normal and neoplastic hematolymphoid tissue through the use of immunohistochemistry and immunofluorescence on tissue sections and cell suspensions.

Materials and Methods

Tissue Samples

Formalin-fixed, paraffin-embedded tissue samples were obtained from the archives of the Department of Pathology, Stanford University Medical Center (SUMC). Tissue microarrays (TMAs) incorporating SUMC pathological material were constructed as described previously.[25] TMAs encompassing 265 cases of classical Hodgkin lymphoma from the Federal University of Rio de Janeiro were also used.[26] All tissues were obtained before treatment, and Institutional Review Board approval was obtained for the study. For expression in normal hematopoietic tissue, whole-tissue sections of normal human spleen, thymus, bone marrow, and tonsil were used. Hematolymphoid neoplasia was classified according to the current, 2008 World Health Organization (WHO) classification.[27]

Immunohistochemistry

TMAs, whole sections of human lymphoma and leukemia samples, and normal human hematopoietic tissue samples were sectioned at 0.4-μm thickness, deparaffinized in xylene, and hydrated in graduated alcohols. Slides were pretreated with 10 mmol/L citrate buffer (pH 6.0). Slides were then stained with anti-CD137 antibody (clone BBK-2; Thermo Scientific, Fremont, CA) at 1:30 dilution. Multiple different commercially available antibodies were tested, but the most consistent staining results were ob-

tained with clone BBK-2. Slides were developed using the Dako Envision method (Dako, Carpinteria, CA) and were coverslipped with aqueous-based mounting medium. Immunohistologic staining for CD20 (L26, dilution 1:1000; Dako), CD3 (rabbit polyclonal, dilution 1:50; Cell Marque, Rocklin, CA), CD21 (IF8, dilution 1:20; Dako), CD23 (1B12, dilution 1:50; Novocastra Laboratories, Newcastle upon Tyne, UK), CD4 (IF6, dilution 1:20; Novocastra Laboratories), CD8 (C8/144B, dilution 1:200; Dako), CD30 (BerH2, dilution 1:40; Dako), CD15 (Carb3, dilution 1:250; Dako), and ALK1 (clone ALK1, dilution 1:75; Dako) were performed on a BenchMark XT (Ventana Medical Systems, Tucson, AZ), or a Leica Bond-Max (Leica Microsystems, Buffalo Grove, IL; Wetzlar, Germany) automated immunostainer, using standard retrieval conditions. *In situ* hybridization with the EBER probe was performed using an INFORM EBER kit on a BenchMark XT immunostainer (Ventana Medical Systems). Stains for CD21 and CD23 were performed on sections of normal tonsil and histiocytic/dendritic cell neoplasms, whereas stains for CD30, CD15, and EBER were performed on a TMA composed of classical Hodgkin lymphoma cases. The remaining immunohistochemical stains (CD3, CD4, CD8, CD20, and ALK1) were performed to further characterize select T-cell lymphoma cases. TMAs were scored as 0 = no staining, 1 = uninterpretable (loss of sample tissue or high background), 2 = weak staining (5% to 20% of cells positive), and 3 = strong staining (>20% of cells positive). For the immunohistochemistry data on tumor cell expression of CD137 (Table 1), cases were scored positive if >5% of the lesional cells stained positive for CD137. Each sample was independently scored by three pathologists (Y.N., M.W.A., and A.G.F.), with a concordance rate of 98%. Discrepant cases were jointly reviewed using a multiheaded microscope and a final score was assigned.

Immunofluorescence

Paraffin-embedded whole-tissue sections of normal human tonsil (*n* = 12), classical Hodgkin lymphoma (*n* = 10), peripheral T-cell lymphoma not otherwise specified (PTCL NOS) (*n* = 3), and angioimmunoblastic T-cell lymphoma (AITL) (*n* = 1) were sectioned at 0.4-μm thickness and deparaffinized; microwave-induced antigen retrieval was performed in 0.1 mol/L sodium citrate (pH 6.0). Slides were then stained with mixtures of two of three primary antibodies [CD137 (mouse anti-human) (1:30 dilution; Thermo Scientific, Fremont, CA), CD3 (rabbit anti-human) (1:100 dilution; Cell Marque), and Oct-2 (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA)]. Slides were then washed in PBS (pH 7.5) and incubated in the dark for 30 minutes with a mixture of two secondary antibodies: goat anti-mouse IgG labeled with Alexa Fluor 568 and goat anti-rabbit IgG labeled with Alexa Fluor 488 (Invitrogen, Carlsbad, CA). Slides were then washed in PBS (pH 7.5) and counterstained by incubation with Vectashield DAPI (Vector Laboratories, Burlingame, CA). The slides were then coverslipped with an aqueous-based mounting medium.

Table 1. Tumor Cell Expression of CD137 Protein Determined by Immunohistochemistry

Tumor subtype	Total positive (n/N)	% Positive
Histiocytic and dendritic cell neoplasms (n = 23)	6/23	26
Follicular dendritic cell sarcoma	6/7	86
Interdigitating dendritic cell tumor	0/1	0
Langerhans cell histiocytosis	0/10	0
Rosai-Dorfman disease	0/3	0
Histiocytic sarcoma	0/2	0
Hodgkin lymphoma (n = 225)	179/225	80
Classical Hodgkin lymphoma (CHL)	179/208	86
Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)	0/17	0
T-cell lymphoma (n = 80)	46/80	58
T-lymphoblastic lymphoma/leukemia	0/7	0
Peripheral T-cell lymphoma, not otherwise specified (PTCL NOS)	9/16	56
Angioimmunoblastic T-cell lymphoma (AITL)	18/32	56
Mycosis fungoides	6/7	86
Large T-cell lymphoma, transformed from mycosis fungoides	7/9	78
Subcutaneous panniculitis-like T-cell lymphoma	1/1	100
Anaplastic large cell lymphoma (ALK$^+$ and ALK$^-$)	5/8	63
Extranodal NK/T-cell lymphoma, nasal type	34/93	37
B-cell lymphoma (n = 359)	12/359	3
Follicular lymphoma	0/134	0
Grade 1 and 2	0/84	0
Grade 3A and 3B	0/50	0
Diffuse large B-cell lymphoma (DLBCL)	0/95	0
Primary mediastinal large B-cell lymphoma (PMBL)	7/46	15
B-cell lymphoma, unclassifiable (features intermediate between DLBCL and CHL)*	5/10	50
Burkitt's lymphoma	0/3	0
Extranodal marginal zone lymphoma	0/10	0
Splenic marginal zone lymphoma	0/5	0
Nodal marginal zone lymphoma	0/3	0
Mantle cell lymphoma	0/12	0
Chronic lymphocytic leukemia/small cell lymphocytic leukemia (CLL/SLL)	0/30	0
Lymphoplasmacytic lymphoma	0/4	0
B-lymphoblastic lymphoma/leukemia	0/5	0
Post-transplant lymphoproliferative disorder	0/2	0
Plasma cell neoplasms (n = 158)	0/158	0
Monoclonal gammopathy of undetermined significance (MGUS)	0/8	0
Multiple myeloma	0/131	0
Plasma cell leukemia	0/11	0
Plasmacytoma	0/8	0
Other (n = 17)		
Systemic mast cell disease	0/2	0
Acute myeloid leukemia (various WHO subtypes)	0/11	0
Blastic plasmacytoid dendritic cell neoplasm	1/4	25

*Referred to in the text as BCLU/DLBCL/CHL.

Flow Cytometry

Expression of CD137 on the surface of lymphoid cells from normal tonsil (n = 2) and from T-cell lymphoma samples (n = 4) was determined using antibodies against CD20 FITC, CD3 Pacific Blue, CD4 PerCP, CD8 APC-H7, CD45RO PE-Cy7, and CD137 PE (Pharmingen; BD Biosciences, San Jose, CA), according to standard protocols for surface staining. Cells were interrogated on an LSR II flow cytometer using FACSDiva software (BD Biosciences), and data were analyzed via Cytobank web-based software (*http://www.cytobank.org*).

Data Analysis and Visualization

Images of normal human hematolymphoid tissue and TMA immunohistochemical staining results were acquired using a Nikon Eclipse E1000 microscope (Nikon, Tokyo, Japan) equipped with 4×, 10×, 20×, 40×, and 60× objective lenses with numerical apertures ranging from 0.05 to 0.90. Images were captured with a SPOT Flex mosaic digital camera (imaging area, 15.2 × 15.2 mm) and SPOT Basic software (SPOT Imaging Solutions; Diagnostic Instruments, Sterling Heights, MI). Immunofluorescence images were acquired using a Nikon Eclipse E800 microscope and a DXM1200C Nikon digital camera. Digitized images were processed using Adobe Illustrator software (Adobe Systems, San Jose, CA). Confocal images were obtained using a Nikon D-Eclipse C1 confocal system and processed with Nikon EZ-C1 3.90 software and Adobe Photoshop software. The stained lymphoma TMA slides were scanned and stored as high-resolution images using an automated slide scanner (Bacus Laboratories; Olympus, Center Valley, PA). Images of primary TMA staining data are available online (*http://tma.stanford.edu/tma_portal/CD137*).

Results

CD137 Protein Is Expressed by FDCs in Normal Hematolymphoid Tissue

To define the expression pattern of CD137 protein in normal human lymphoid tissue, we performed immunofluorescence microscopy on paraffin-embedded tissue sections of normal tonsil. Antibodies to CD3 and the B-cell transcription factor OCT-2 highlighted a normal tonsillar immunoarchitecture of T-cell-rich interfollicular zones and B cells localized predominantly to the GC and mantle zone (Figure 1, A and B). Antibody to CD137 highlighted spindled-appearing cells localized exclusively to the GC, morphologically consistent with FDCs (Figure 1, A and B, insets). Although rare T cells in the interfollicular zones coexpressed CD137, we did not observe expression of CD137 by either GC T cells (Figure 1A, inset) or by OCT-2-expressing GC B cells (Figure 1B). Consistent with prior reports,[5,7] these results suggested that CD137 expression is restricted to FDCs in normal human tonsil. To confirm the expression of CD137 by FDCs, sections of normal tonsil were also stained with
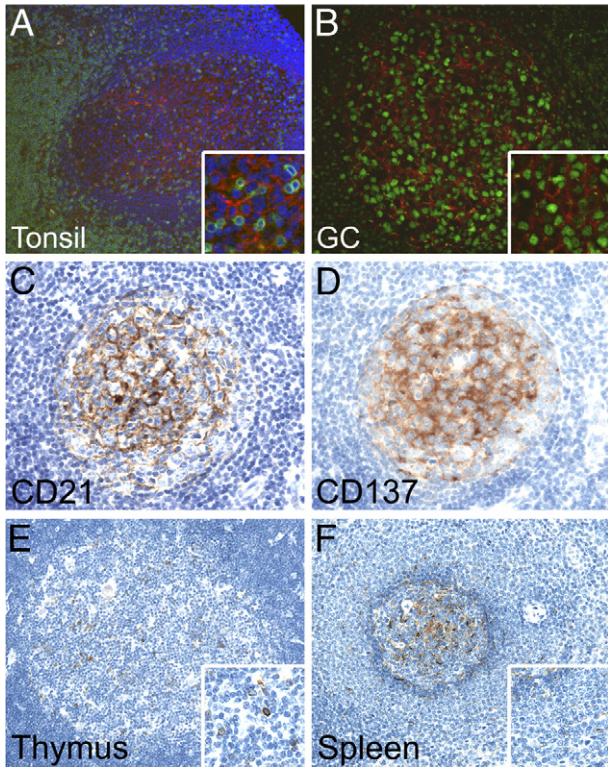
Figure 1. Expression of CD137 protein in normal human hematopoietic tissues. **A:** Section of normal reactive human tonsil stained with antibodies to CD137 (red; cytoplasm and cell membrane) and CD3 (green; cell membrane). DAPI (blue) was used as a nuclear counterstain. **B:** Section of normal reactive human tonsil stained with antibodies to CD137 (red) and OCT-2 (green; nuclear). **C:** Section of normal human tonsil stained with antibody to CD21. **D:** Section of normal human tonsil stained with antibody to CD137. **E:** Section of normal human thymus stained with anti-CD137 antibody. **F:** Section of normal human spleen stained with anti-CD137 antibody. Original magnification: ×100 (**A**); ×400 (**B–D**); ×200 (**E** and **F**); ×600 (**insets**).

antibodies against CD21, a well-characterized marker of FDCs.[28] Indeed, tonsillar GCs showed identical patterns of immunohistochemical reactivity using antibodies to either CD137 or CD21, suggesting that CD137 is a specific marker of FDCs (Figure 1, C and D).

Next, we investigated CD137 protein expression in normal human thymus, spleen, and bone marrow using immunohistochemistry. In normal human thymus, only rare cells within the thymic medulla expressed CD137 protein (Figure 1E). No expression of CD137 was detected in the thymic cortex, suggesting that neither immature T cells nor thymic epithelial cells express CD137. In normal human spleen, CD137 expression was restricted to spindled cells with morphological features of FDCs within GCs of the white pulp (Figure 1F); CD137 expression was not detected within the mantle zones or red pulp. Sections of normal human bone marrow showed an absence of CD137 staining in hematopoietic precursors and in bone marrow stromal elements (data not shown).

To evaluate the expression of CD137 in nonhematolymphoid tissue, a TMA containing samples from 61 cases of both normal and neoplastic tissues derived from human skin, soft tissue, placenta, skeletal muscle, heart, adrenal gland, salivary gland, breast, ovary, uterus, pros-

tate, bladder, colon, stomach, pancreas, lung, kidney, liver, parathyroid gland, thyroid gland, testis, and brain was stained for CD137. No significant CD137 expression was observed in any of these nonhematopoietic tissues (see Supplemental Table S1 at *http://ajp.amjpathol.org*). Although previous reports identified CD137 expression in intratumoral blood vessels,[23] we did not detect significant CD137 expression by endothelial cells in either neoplastic or normal tissue.

CD137 Is Expressed by Follicular Dendritic Cell Neoplasms

Dendritic and histiocytic cell tumors are a heterogeneous group of neoplasms that are often difficult to diagnose solely by morphological criteria. Because CD137 was strongly expressed by normal FDCs, we investigated the expression of CD137 across a diverse group of histiocytic and dendritic cell neoplasms by immunohistochemistry. We found strong and consistent cytoplasmic and cell membrane expression of CD137 in FDC neoplasms, confirming that CD137 expression is maintained during neoplastic transformation (Figure 2A and Table 1). In our series, an equivalent number of cases were positive for
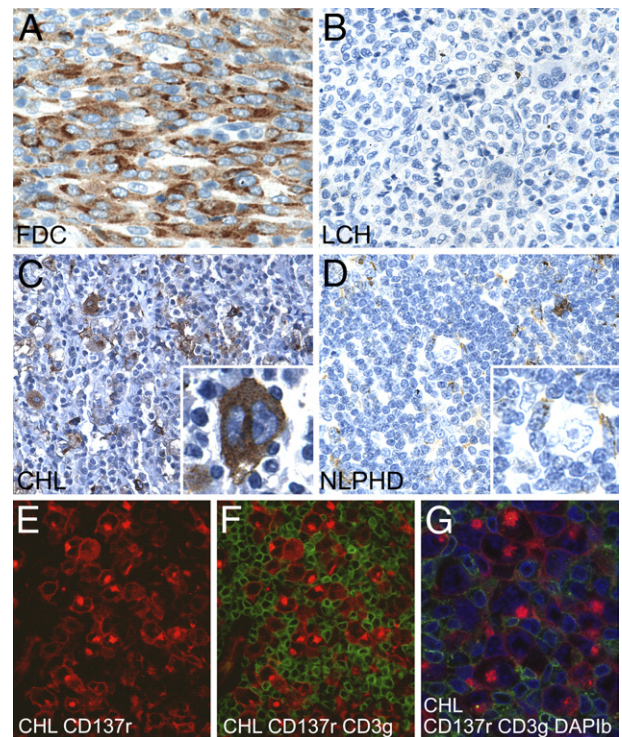
Figure 2. CD137 is expressed by FDC tumors and classical Hodgkin lymphoma. **A–D:** Immunohistochemical stain for CD137 on paraffin-embedded tissue sections of FDC tumor (**A**), Langerhans cell histiocytosis (LCH) (**B**), classical Hodgkin lymphoma (CHL) (**C**), and nodular lymphocyte predominant Hodgkin lymphoma (NLPHD) (**D**). **E** and **F:** Sections of classical Hodgkin lymphoma stained with antibodies to CD137 (red; cytoplasm and cell membrane) and CD3 (green; cell membrane); DAPI (blue) was used as a nuclear counterstain. **G:** Section of classical Hodgkin lymphoma stained with antibodies to CD137 (red; cytoplasm and cell membrane) and CD3 (green; cell membrane), with DAPI (blue) as a nuclear counterstain, visualized using confocal microscopy. Original magnification: ×400 (**A–G**); ×900 (**inset, C**); ×600 (**inset, D**).

Table 2.   Immunohistochemical Characterization of Follicular Dendritic Cell Tumors

Case	CD137	CD21	CD23	CD35
1	+	+	+ (weak)	n/d
2	+	+ (focal)	−	+
3	+	−	+	+ (weak)
4	+	+	n/d	+
5	+ (weak)	+	n/d	n/d
6	−	+	−	−
7	+	+	+	+

n/d, not done.

CD21 (6/7, or 86%) (Table 2). In one case, CD137 was positive but CD21 and CD23 were focal or negative, suggesting that CD137 may be a useful adjunct marker to diagnose FDC tumors in the absence of CD21 and CD23 staining. In contrast, CD137 expression was not observed in other histiocytic neoplasms, including Langerhans cell histiocytosis (Figure 2B) and interdigitating dendritic cell tumor (Table 1). Other spindle cell neoplasms, such as dermatofibrosarcoma protuberans and leiomyosarcoma, failed to stain with CD137 (see Supplemental Table S1 at *http://ajp.amjpathol.org*). One case of blastic plasmacytoid dendritic cell tumor showed weak positive staining for CD137 (Table 1). CD137 was not expressed in any subtype of acute myeloid leukemia that was evaluated (Table 1). Reactive histiocytic proliferations such as Rosai-Dorfman disease also failed to express CD137 (Table 1).

CD137 Is a Marker of Classical Hodgkin Lymphoma

TMAs and whole-tissue sections were used to investigate the expression of CD137 in both classical Hodgkin lymphoma (CHL, 208 cases) and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL, 17 cases). We found expression of CD137 protein in the Hodgkin and Reed-Sternberg cells of 179/208 (86%) CHL cases (Figure 2C and Table 1). By immunohistochemistry, CD137 staining was localized to the membrane and cytoplasm, often with punctate paranuclear staining similar to the typical pattern of reactivity of CD15 (Figure 2C). CD137 was also expressed in a considerable proportion of cases that lacked CD15 staining (63/80, or 79%), suggesting that CD137 is a useful additional marker for CHL diagnosis with lacking CD15 expression (see Supplemental Table S2 at *http://ajp.amjpathol.org*). CD137 was frequently coexpressed with CD30 (176/197, 89%), but CD137 expression did not correlate with the presence of Epstein-Barr virus (EBV) as identified by *in situ* hybridization for EBER RNA ($P = 0.98$). The expression of CD137 did not correlate with overall survival ($P = 0.504$) or progression-free survival ($P = 0.235$) in our series of cases (see Supplemental Figures S1 and S2 at *http://ajp.amjpathol.org*). In cases of NLPHL, CD137 highlighted the expanded and disrupted FDC networks that are often prominent in this diagnosis (Figure 2D), but the atypical lymphocyte predominant cells (Figure 2D, inset) failed to stain for CD137. Immunofluorescence staining of CD137

showed crisp membranous localization with faint cytoplasmic and strong paranuclear staining when visualized using either standard (Figure 2, E and F) or confocal microscopy (Figure 2G). Very little CD137 staining was observed in the background cellular infiltrate, including infiltrating T lymphocytes (Figure 2, F and G).

CD137 Expression by Non-Hodgkin B-Cell Lymphoma Tumor Cells

We next asked whether CD137 was expressed by other tumors of the lymphoid lineage, in a broad survey of 359 non-Hodgkin B-cell lymphomas and 158 plasma cell neoplasms (Table 1). In contrast to the frequent expression of CD137 in CHL, no significant CD137 expression was detected in immature B-cell tumors or the majority of mature B-cell lymphomas, including follicular lymphoma (Figure 3A), DLBCL (Figure 3B), and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL; Figure
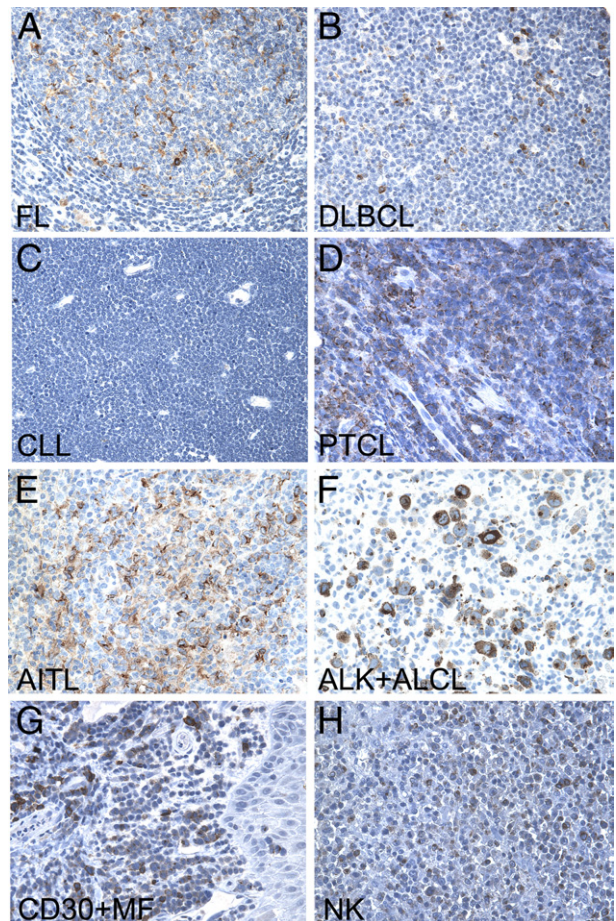
Figure 3. CD137 expression by B-, T-, and NK-cell-derived lymphomas. Immunohistochemical stains for CD137 on paraffin-embedded tissue sections of follicular lymphoma (FL) (**A**), diffuse large B-cell lymphoma (DLBCL) (**B**), and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL) (**C**) show lack of expression of CD137 in tumor cells. In contrast to B-cell lymphomas, peripheral T-cell lymphoma not otherwise specified (PTCL) (**D**), angioimmunoblastic T-cell lymphoma (AITL) (**E**), ALK$^+$ anaplastic large cell lymphoma (ALCL) (**F**), CD30$^+$ mycosis fungoides (MF) (**G**), and extranodal NK/T-cell lymphoma, nasal type (NK) (**H**), show tumor cell staining for CD137. Original magnification, ×400.

3C). CD137 was also not expressed by the tumor cells of marginal zone lymphoma, mantle cell lymphoma, or plasma cell neoplasms (Table 1). In contrast, we observed CD137 expression in a subset of cases of primary mediastinal large B-cell lymphoma (PMBL) (7/46, or 15%; Table 1). Of note, CD137 staining in the positive cases of PMBL highlighted large atypical Hodgkin-appearing cells but not all lymphoma cells within the biopsy specimen. Because PMBL and CHL share overlapping morphological, immunophenotypic, and molecular features, we stained an additional 10 cases of B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma (BCLU/DLBCL/CHL), a new WHO 2008 diagnostic category that was created to formally recognize and better define cases exhibiting overlap between those two categories (the so-called gray-zone lymphoma).[29,30] Notably, we observed CD137 expression in 5 of 10 (50%) of these cases (Table 1; see also Supplemental Figure S3 at *http://ajp.amjpathol.org*).

Although CD137 was not found in the majority of B-cell lymphomas, CD137-expressing cells were detected within the tumor microenvironment. The number of CD137^{+} cells was generally low, representing ≤10% of the total tissue cellularity. Among DLBCL cases, 62% showed CD137^{+} infiltrating cells, possibly representing infiltrating T cells or other stromal components. Among follicular lymphomas, 72% of cases showed CD137^{+} cells within the neoplastic follicles, with residual FDC networks contributing to the overall staining pattern. Among cases of marginal zone and mantle cell lymphoma, a similar proportion (61% and 50%, respectively) showed CD137^{+} cells within the microenvironment, but significantly fewer cases (17%) of CLL/SLL showed CD137^{+} cells in the microenvironment (data not shown). The absence of CD137 expression in tumor B cells of these non-Hodgkin B-cell lymphoma was confirmed also by flow cytometry (data not shown).

CD137 Is Expressed by a Subset of T-Cell and NK/T-Cell Lymphomas

In contrast to non-Hodgkin B-cell lymphomas, approximately 58% of T-cell lymphomas showed immunoreactivity for CD137. CD137 was expressed across multiple histological subtypes of mature T-cell lymphomas, including PTCL NOS (Figure 3D), AITL (Figure 3E), ALK[+] anaplastic large cell lymphoma (Figure 3F), and mycosis fungoides (Figure 3G). CD137 was not expressed by immature T-cell lymphomas. Double-immunofluorescence labeling of select PTCL and AITL cases confirmed coexpression of CD3 and CD137 on the lesional cells (see Supplemental Figure S4 at *http://ajp.amjpathol.org*). Approximately 37% of cases of extranodal NK/T-cell lymphoma, nasal type, also expressed CD137 (Figure 3H). CD137 staining of NK/T-cell neoplasms tended to be less intense than CHL or T-cell lymphoma.

Although we observed tumor cell expression of CD137 by immunohistochemistry, we next asked whether we could detect cell-surface expression of CD137 on unfixed

tumor cells by flow cytometry, to ascertain the possible efficacy of anti-CD137 antibody therapy or risk for promoting tumor growth with agonistic anti-CD137 antibody therapy; the latter antibody is currently in clinical trials as BMS-663513 (*http://www.clinicaltrials.gov*). Flow cytometry confirmed that CD137 is indeed expressed on the cell surface as illustrated in a case of ALK[−] anaplastic large cell lymphoma (Figure 4). This T-cell lymphoma showed effacement of the lymph node architecture by an atypical and a pleomorphic large cell infiltrate associated with eosinophilia and a prominent vascular proliferation. By immunohistochemistry, the tumor cells expressed CD3, CD8, CD30 (diffuse and strong expression), and CD137, but lacked expression of CD20, CD4, CD15, and ALK1 (Figure 4, A–F). By flow cytometry gating on the large-cell subset to enrich for the neoplastic cells, approximately 18% of the CD3+CD8+ tumor cells expressed CD137 (Figure 4G), confirming the presence of CD137 protein on the tumor cell membrane.

Discussion

The expression of CD137 protein in both normal and neoplastic human lymphoid tissue is important to evaluate, because its cell- and tissue-specific expression patterns are likely to influence the choice and usage of
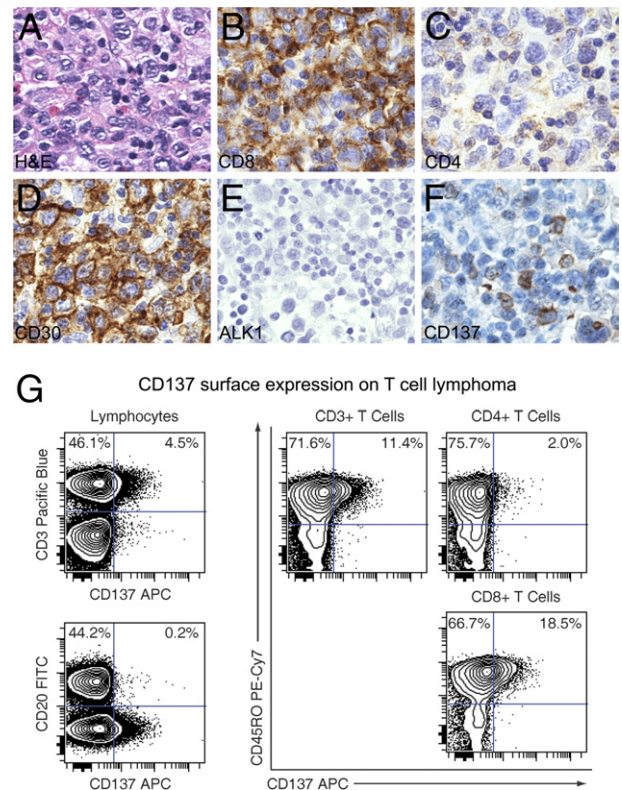
Figure 4. Detection of tumor cell CD137 expression by flow cytometry. **A–F:** A case of ALK[−] anaplastic large cell lymphoma was stained with H&E (**A**) and antibodies to CD8 (**B**), CD4 (**C**), CD30 (**D**), ALK1 (**E**), and CD137 (**F**). The lymphoma cells showed positivity for CD8, CD30, and CD137. **G:** Flow cytometric evaluation of CD137 expression showed lack of CD137 expression by CD20^{+} B cells and CD4^{+} T cells, but detectable expression of CD137 in a proportion (18.5%) of CD8^{+} tumor T cells. Original magnification, ×600.

anti-CD137 immunomodulatory therapies. In normal tonsil and lymph node, we found that CD137 is a specific marker of FDCs. In contrast, we observed very little expression of CD137 protein by T and B cells within normal tonsil, spleen, and thymus. Pauly et al[7] found a similar pattern of CD137 reactivity in normal human lymph nodes using the same monoclonal antibody on frozen tissue sections. Thus, the specificity of CD137 reactivity we observed in the present study is not an artifact of tissue processing. Such a restricted pattern of CD137 protein expression supports the hypothesis that CD137 is likely to be an important regulator of FDC and B-cell interactions during the GC reaction.[7] CD137 expression by FDCs could therefore also be considered a part of the normal GC signature, along with B-cell-specific markers such as LMO2, BCL-6, and CD10 and T-cell-specific markers such as PD-1 and CXCL13.

We also demonstrated that CD137 is a highly specific immunohistochemical marker of neoplastic FDCs. FDC tumors are uncommon hematopoietic neoplasms and are often overlooked in the histological differential diagnosis of spindle-cell tumors. CD137 strongly stained the majority of FDC tumors in our series, indicating that CD137 immunohistochemistry is a useful diagnostic marker of FDC tumors (along with CD21, CD23, and podoplanin).[31,32]

Perhaps the most novel finding of the present study was the expression of CD137 in a significantly high proportion of CHL samples. Although CD137 expression has been previously detected in cultured CHL cells,[33] to our knowledge this is the first report describing CD137 expression in primary CHL samples. CD137 immunohistochemistry may be particularly helpful for cases of CHL with weak or absent expression of the typical diagnostic markers (CD30, CD15, and paired box protein Pax-5). In such cases, the differential diagnosis includes entities such as NLPHL, CD30^{+} B-cell lymphoma, and T-cell lymphoma, each with a markedly different prognosis and treatment. Although CD137 expression may not entirely exclude T-cell lymphoma from the differential diagnosis, our results suggest that CD137 would serve as an excellent adjunct marker on the immunohistologic panel used to rule out a diagnosis of NLPHL. Among large B-cell lymphomas, the expression of CD137 in a subset of PMBL and the new WHO 2008 category of BCLU/DLBCL/CHL further underscores the molecular overlap among PMBL, CHL, and BCLU/DLBCL/CHL. Although CD137 expression does not appear to resolve the diagnostic dilemma posed by gray-zone B-cell lymphomas of the mediastinum, its expression may represent a novel therapeutic strategy for the management of these tumors, particularly BCLU/DLBCL/CHL tumors, which typically exhibit a poor clinical outcome.[34] Although CD137 is a useful diagnostic marker for CHL, in our series of cases it was not significantly associated with overall survival or progression-free survival.

Although there is much evidence to support the GC B-cell derivation of CHL,[35,36] a subset of CHL cases have also been reported to express T-cell markers[37,38] and FDC markers.[39] Although the expression of non-B-cell antigens may be a consequence of transcriptional dys-

regulation during neoplastic transformation,[40] another possibility is that CD137 expression by CHL is evidence of heterotopic intracellular fusion between FDCs and GC B cells. This hypothesis would explain the frequent finding of binucleated (Hodgkin/Reed-Sternberg) cells in CHL and the relative lack of expression of B-cell markers such as CD20. However, experiments designed to detect molecular evidence of cell fusion events in CHL suggest that binucleated CHL cells derive from endomitosis, rather than from cellular fusion.[41]

CD137 is also expressed in a significant proportion of T- and NK-cell-derived lymphomas. In the present study, CD137 expression was not specific for the histological subtype of mature T-cell lymphoma, consistent with the broad expression pattern of CD137 by normal mature T-cell subsets.[11] We also observed CD137 expression in a significant proportion of cases of extranodal NK/T-cell lymphoma, nasal type, although the staining was generally weaker in intensity. Although the effect of CD137 signaling to promote T-cell proliferation and survival is well studied, much less is known about the biological role of CD137 expression by normal human NK cells. Further experiments are required to address the functional significance of CD137 expression by either T-cell or NK-cell neoplasms.

Consistent with the lack of expression of CD137 by normal B cells, we did not observe CD137 expression by the majority of non-Hodgkin B-cell lymphomas. However, we did observe that a significant proportion of B-cell lymphomas showed increased numbers of infiltrating CD137^{+} cells in the tumor microenvironment. Although it is difficult to phenotype these cells using immunohistochemistry, members of our research group have identified increased numbers of CD137^{+} T cells in follicular lymphoma, mantle cell lymphoma, and DLBCL using flow cytometry with a different anti-CD137 monoclonal antibody (clone 4B4-1).[18] FDCs are difficult to analyze using flow cytometry, but our immunohistochemical data suggest that FDCs may also contribute to the expression of CD137 in the microenvironment of B-cell lymphomas. Further experiments are required to address the role of CD137/CD137L interactions in the tumor microenvironment, to fully understand the role of this receptor-ligand pair in B-cell neoplasia.

Our observation that select subtypes of lymphoma express CD137 has significant implications for the use of anti-CD137 immunotherapy. To date, only agonistic anti-CD137 monoclonal antibodies have been tested in preclinical models and small phase 1 clinical trials.[42] In this therapeutic strategy, stimulation of CD137 on T cells via agonistic anti-CD137 antibody results in T-cell activation, proliferation, and enhancement of anti-tumor immunity. However, our observation that CHL, FDC tumors, T-cell lymphoma, extranodal NK/T-cell lymphoma, PMBL, and BCLU/DLBCL/CHL can all express CD137 raises the possibility that these tumor subtypes could be directly targeted by anti-CD137 antibodies. Although humanized antagonistic anti-CD137 monoclonal antibodies have been developed,[43,44] to our knowledge these reagents have not been developed as possible therapeutic agents. Perhaps this is not surprising, given the lack of

convincing evidence that CD137 is expressed by primary human solid-organ tumors.[23,45] Detection of CD137 on the cell membranes of unfixed neoplastic T cells by flow cytometry is encouraging in that CD137 could indeed function as a therapeutic target in patients with lymphoma subtypes that express CD137. Additional experiments are required to assess whether CD137 represents a useful therapeutic target on patients with CD137+ lymphoma subtypes.

Acknowledgments

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