AP1-Dependent Galectin-1 Expression Delineates Classical Hodgkin and Anaplastic Large Cell Lymphomas from Other Lymphoid Malignancies with Shared Molecular Features

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Abstract

Purpose: Galectin-1 (Gal1) is an immunomodulatory glycan-binding protein regulated by an AP1-dependent enhancer in Hodgkin Reed-Sternberg cells. We recently found that Reed-Sternberg cell Gal1 promotes the immunosuppressive T-helper 2/T-regulatory cell – skewed microenvironment in classical Hodgkin lymphoma (cHL). We sought to investigate whether the coordinate expression of activated AP1 pathway components and Gal1 serves as a diagnostic signature of cHL. In addition, because there are common signaling and survival pathways in cHL and additional non – Hodgkin lymphomas, we also evaluated whether the AP1/Gal1 signature is shared by other molecularly or morphologically related lymphomas.

Experimental Design: We evaluated 225 cases of primary cHL and non – Hodgkin lymphoma for evidence of a functional AP1/Gal1 signature by immunohistochemical techniques.

Results: Gal1 is selectively expressed by malignant Reed-Sternberg cells in >90% of primary cHLs, and Gal1 expression is concordant with the activated AP1 component, c-Jun. In contrast, diffuse large B-cell lymphoma, primary mediastinal large B-cell lymphoma, and another Hodgkin-related entity, nodular lymphocyte – predominant Hodgkin lymphoma, do not express Gal1. However, anaplastic large cell lymphoma (ALCL), consistently expresses both Gal1 and its transcriptional regulator, c-Jun. The presence of activated c-Jun, indicative of functional AP1 activity, was confirmed by phospho-c-Jun immunostaining in cHL and ALCL.

Conclusions: These findings establish a functional AP1 signature that includes Gal1 expression in cHL and ALCL and suggests a common mechanism for tumor immunotolerance in these diseases. In addition, the combination of Gal1 and c-Jun serve as diagnostic biomarkers that delineate cHL and ALCL from other lymphomas with shared morphologic and/or molecular features.

Galectins, a family of highly conserved glycan-binding proteins, are characterized by their ability to recognize *N*-acetyllactosamine sequences, which can be displayed on both *N*- and *O*-glycans on cell surface glycoconjugates (1, 2). Galectin-1 (Gal1), a prototype member of this family, has emerged as a regulator of T-cell survival, homeostasis, and inflammation (2–4). In murine models of autoimmunity, Gal1 suppresses T helper (Th)-1 and Th-17–mediated responses and skews the immune response toward a Th2 profile (5–8). A mechanistic analysis of these selective immunosuppressive effects recently revealed that Th1 and Th17 effector cells express the repertoire of cell surface glycans that are critical for Gal1 binding and subsequent cell death, whereas Th2 cells are protected from Gal1 through differential sialylation of cell surface glycoproteins (8). Interestingly, selective blockade of Gal1 in tumor tissue results in increased Th1-mediated antitumor responses *in vivo*, suggesting a potential role of this protein in tumor-immune escape (9). These findings were validated in human tumors, demonstrating an inverse correlation between Gal1 expression and the frequency of tumor infiltrating T cells (10).

Classical Hodgkin lymphomas (cHL; ref. 11) are unusual tumors with small numbers of malignant RS cells within an extensive inflammatory infiltrate, which includes abundant Th2 and T regulatory cells (12). Until recently, the factors that are responsible for creating and maintaining the Th2/T regulatory–skewed immunosuppressive microenvironment in cHL were undefined.

We recently reported that Hodgkin lymphoma cell lines selectively overexpress Gal1 via an AP1-dependent enhancer and constitutive activation of the AP1 transcription complex (13). In *in vitro* coculture assays, cHL Gal1 promoted the survival and expansion of Th2 cells at the expense of Th1 cells (13). In *in vitro* assays, Gal1 also favored the retention and/or expansion of T regulatory cells. In a pilot series of primary cHLs,

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Gal1 was selectively overexpressed by the malignant Reed-Sternberg cells (RS cells; ref. 13). Together, these observations indicate that the AP1-dependent production of Gal1 fosters the immunosuppressive microenvironment in cHL.

Herein, we ask whether the coordinate expression of activated AP1 pathway components and Gal-1 serves as a diagnostic signature of cHL. Because recent studies have revealed the unexpected presence of common signaling and survival pathways in cHL and additional non–Hodgkin lymphomas, we also sought to determine whether the AP1/Gal1 signature is shared by other molecularly or morphologically related lymphomas. In particular, we examined nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), which exhibits morphologic similarities to cHL, including rare tumor cells within an abundant inflammatory infiltrate but has a distinct clinical course and therapeutic options (14). We also evaluated primary mediastinal large B-cell lymphoma (MLBCL), a B-cell tumor with molecular similarities to cHL including a dependence upon nuclear factor-κB signaling for growth and survival (15) and the more common diffuse large B-cell lymphoma (DLBCL). In addition, we evaluated anaplastic large cell lymphoma (ALCL), an aggressive T-cell tumor with certain morphologic similarities to cHL and a shared reliance upon AP1 signaling (16).

Materials and Methods

Case selection. Cases were derived from the files of Brigham & Women's Hospital with Institutional Review Board approval. All diagnoses were established at the time of the original biopsy evaluation and based on the criteria established by the current WHO classification system (11). Diagnoses were subsequently confirmed during review of each case as part of this study.

Immunohistochemistry. Immunohistochemistry was performed using 5-μm-thick formalin- or B5-fixed, paraffin-embedded whole tissue sections on individual slides or tissue microarrays as previously described (17). Briefly, slides were soaked in xylene, passed through graded alcohols, and then pretreated with 10-mmol/L citrate (pH 6.0; Zymed) in a steam pressure cooker (Decloaking Chamber; BioCare Medical) as per manufacturer's instructions. All further steps were done at room temperature in a hydrated chamber. Slides were then treated with Peroxidase Block (DAKO USA) for 5 min to quench endogenous peroxidase activity. Unless otherwise stated, the Gal1 antibody used for all studies is a primary rabbit polyclonal anti-Gal1 antibody generated in the laboratory of G.A.R. and previously established to be specific for Gal1 (9, 13, 18–21). This antibody was applied at 1:10,000 dilution for 1 h in DAKO diluent. In a subset of cases, tumor staining for Gal1 was confirmed with both a distinct rabbit polyclonal antisera generated in the laboratory of M.A.S. against full-length recombinant Gal1, and a commercially available rabbit polyclonal antibody (PeproTech, Inc.) by the method previously described (ref. 22; data not shown). For detection of AP1 signaling, primary rabbit monoclonal anti–c-Jun (1:50 dilution; clone 60A8; Cell Signaling Technology), or primary rabbit monoclonal anti–phospho-c-Jun specific for phosphorylated serine at amino acid position 63 (1:50 dilution; clone 54B3; Cell Signaling Technology) was applied in DAKO diluent (DAKO) for 1 h at room temperature. Slides were washed in 50 mmol/L Tris-Cl (pH 7.4), and anti-rabbit or anti-murine horseradish peroxidase–conjugated antibody solution (Envision+ detection kit; DAKO) was applied for 30 min. After further washing, immunoperoxidase staining was developed using a 3,3′-diaminobenzidine chromogen kit (DAKO) per the manufacturer and counterstained with Harris hematoxylin (Polyscientific).

Immunohistochemical evaluation. Reactivity for Gal1, c-Jun, and phospho-c-Jun for all cases was determined and scored independently by two hematopathologists (S.J.R and J.L.K). Intensity of staining for Gal1 was scored as follows: 0, no staining detected; 1+, weak staining; 2+, moderate staining; 3+, strong staining of the tumor cells. Positive staining for a case was defined as 2+ or 3+ cytoplasmic staining in >50% of the tumor cells. A staining of 0 or 1+ in >50% of tumor cells, or focal reactivity of 2+ or 3+ in <50% of the tumor cells was considered negative. Positive staining of endothelial cells and macrophages served as positive internal controls. Staining for c-Jun and phospho-c-Jun was considered positive if nuclear staining for the antigen was observed >50% and >25% of interphase tumor nuclei, respectively. Staining of endothelial and dendritic cells served as internal positive controls for both antibodies. All cases were photographed at ×1,000 original magnification with an Olympus BX41 microscope with the objective lens of ×100/0.75 Olympus UPlanFL (Olympus). The pictures were taken using Olympus QColor3 and analyzed with acquisition software QCapture v2.60 (QImaging) and Adobe Photoshop 6.0 (Adobe). The differences in Gal1 expression in specific types of lymphoma were evaluated with a Fisher exact test.

Results

Gal1 expression in Hodgkin lymphoma. To determine whether Gal1 expression is a diagnostically useful distinguishing feature of primary cHL, we examined by immunohistochemistry an extensive series of cHLs (72 cases). These primary tumors included the 2 major subtypes of cHL: nodular sclerosis (45 cases) and mixed cellularity (15 cases). In addition, our cohort included several cases diagnosed as cHL not otherwise specified (cHL not otherwise specified, 11 cases) and the rare lymphocyte-rich subtype of cHL (1 case). A subset of cases was also evaluated for evidence of EBV infection by *in situ* hybridization for EBV-encoded RNAs (EBV-encoded RNA+, 10 cases; EBV-encoded RNA-, 33 cases).

Gal1 immunohistochemistry revealed robust cytoplasmic and weak cell membrane staining of RS cells in cHL (Fig. 1A, representative example). Intermixed macrophages, dendritic cells, and at least a subset of endothelial cells also showed expression of Gal1. In contrast, the preponderance of small lymphocytes and occasional granulocytes comprising the majority of the inflammatory infiltrate surrounding the RS cells were negative for Gal1 (Fig. 1A). The majority of primary cHLs (61%) exhibited very strong expression of Gal1 [3+ on a scale of 0 to 3 (no staining to very strong staining, respectively); Fig. 1A]; additional cases (31%) exhibited moderate staining scored as 2+ (Fig. 1B). In only rare cases were cHL RS cells negative for Gal1 expression (Table 1). Overall, 66 of 72 (92%) cases of cHL exhibited moderate to strong expression of Gal1 by the RS cells (Fig. 1A and B; Table 1). All of the examined subtypes of cHL were largely Gal1+; similarly, both EBV+ and EBV- tumors expressed this glycan-binding protein (Table 1).

In contrast to cHLs, none of the tumor cells in cases of NLPHL exhibited moderate or strong (2 or 3+) Gal1 expression (Fisher exact test, $P < 0.000001$; Fig. 1C; Table 1). However, certain NLPHLs (9 of 15 cases, 60%) had weak, focal, perinuclear Gal1 staining. This Gal1 immunohistochemical pattern was only detected in a subset of NLPHLs and not observed in any other lymphoid tumors. These differences in Gal1 expression in cHL and NLPHLs are of considerable interest because NLPHLs share certain morphologic features with cHLs but differ in their prognosis and treatment.

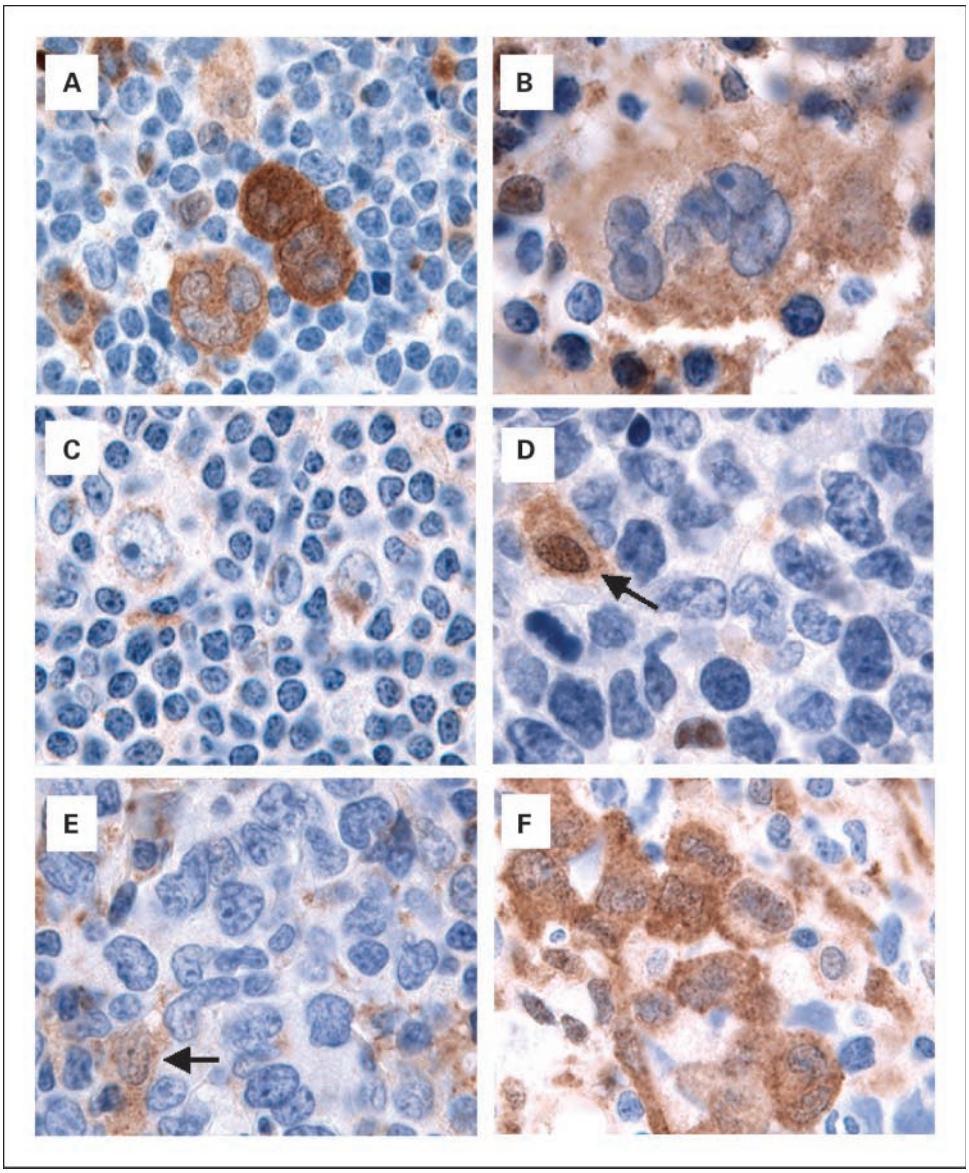

Fig. 1. Primary human tumor samples stained for Gal1 and photographed at ×1,000 magnification. *A,* cHL, with cytoplasmic staining scored as 3+ in the RS cells and variants, *B,* cHL, with cytoplasmic staining scored as 2+ in the RS cells and variants. *C,* NLPHL with weak, perinuclear staining of the Lymphocytic and Histiocytic cells that was scored as negative. *D,* primary mediastinal large B-cell lymphoma with no cytoplasmic staining of tumor cells but cytoplasmic staining of an adjacent macrophage (*arrow*). *E,* diffuse large B-cell lymphoma with no cytoplasmic staining of tumor cells but with weak cytoplasmic staining of an intermixed macrophage (*arrow*). *F,* ALCL with cytoplasmic staining of tumor cells scored as 3+.

Gal1 expression in primary mediastinal and diffuse large B-cell lymphoma. With emerging data indicating that seemingly disparate tumors such as cHL and primary MLBCL share important molecular features and survival pathways (15), we evaluated the expression pattern of Gal1 in MLBCL (n = 17) and compared these findings to nonmediastinal forms of DLBCL (n = 102).

Expression of Gal1 was found in only 2 of 17 cases (12%) of MLBCL (Fig. 1D; Table 1). Similarly, only 7 of 102 cases of DLBCL (7%) exhibited Gal1 expression (Fig. 1E; Table 1). We found no distinguishing clinical, morphologic, or phenotypic features among the rare Gal1-positive MLBCLs or DLBCLs upon review. Positive Gal1 immunostaining is, thus, a powerful marker for distinguishing cHL from MLBCL (P < 0.000001, Fisher exact test) and delineating cHL from DLBCL (P < 0.000001, Fisher exact test).

Gal1 expression in ALCL. ALCLs are aggressive large cell lymphomas of T-cell origin with high levels of c-Jun and JunB expression and constitutive activation of AP1 (23). Given the role of the AP1 pathway in ALCL and the AP1-dependent Gal1 expression in cHL, we next asked whether ALCLs overexpressed Gal1. Ninety-five percent of primary ALCLs (18 of 19 cases) exhibited moderate to strong (2+ or 3+) diffuse cytoplasmic Gal1 staining (Fig. 1F); both ALK+ and ALK- tumors expressed this glycan-binding protein (Table 1).

The polyclonal Gal1 antiserum used for the above studies is well-characterized and known to be specific (13, 18). However, to validate our observations, we did immunostaining on a subset of cases with two additional polyclonal Gal1 antisera (one generated in the laboratory of M.A.S. and one commercially available reagent). There was a perfect correlation of staining patterns in Gal1-positive and Gal1-negative tumors with the two Gal1 antisera generated in the laboratories of our coauthors (data not shown). In this subset of cases, the commercially available antisera that recognizes Gal1 (22) was less sensitive than our polyclonal Gal1 antisera in detecting Gal1 expression in primary tumor cells (data not shown).

Table 1. Galectin-1 Expression in Lymphomas with Large Cell Morphology

Diagnosis	Gal1 positive
cHL-Total*	66/72 (92%) †
NSHL	41/45 (91%)
MCHL	14/15 (93%)
LRHL	1/1 (100%)
cHL, nos	10/11 (91%)
NLPHL ‡	0/15 (0%) †
MLBCL	2/17 (12%) †
DLBCL	7/102 (7%) †
ALCL§	18/19 (95%)

Abbreviations: NSHL, classical Hodgkin lymphoma, nodular sclerosis subtype; MCHL, classical Hodgkin lymphoma, mixed cellularity subtype; LRHL, classical Hodgkin lymphoma, lymphocyte-rich subtype; cHL, nos, classical Hodgkin lymphoma, not otherwise specified.
*8/10 EBV+ cases are Galectin-1 positive; 30/33 EBV- cases are Galectin-1 positive.
† Differential Gal1 expression between cHL and NLPHL, MLBCL, and DLBCL is significant, $P < 0.000001$.
‡ Faint perinuclear staining observed in some cases.
§Two of eleven tested cases ALK-1 positive; 9 of 11 tested cases ALK-1 negative.

Association between Gal1 and c-Jun expression. Given the AP1 dependence of Gal1 in cHL (13) and the selective expression of Gal1 in another AP1-driven tumor, ALCL, we next assessed the coexpression of the AP1 component, c-Jun, and Gal1 in our series of primary lymphomas (Fig. 2).

In agreement with prior reports (23), we found that RS cells of all examined primary cHLs express nuclear c-Jun (Fig. 2B; Table 2). In this extensive series of primary cHLs, all examined Gal1+ tumors expressed high levels of c-Jun (Table 2 and representative example; Fig. 2A and B). Almost all examined primary ALCLs also expressed c-Jun, which localized to the nucleus (Fig. 2E; Table 2). The concordance of Gal1 and c-Jun expression in ALCL (Fig. 2D and E) strongly suggests that c-Jun–mediated signaling could be responsible for near ubiquitous Gal1 expression in this tumor type—a possibility supported by the lack of c-Jun expression in the single ALCL negative for Gal1 (Table 2).

Among other Hodgkin variants and large cell lymphomas, we found that c-Jun expression was uncommon. Eighty-seven percent (13 of 15 cases) of NLPHL, 82% (84 of 102 cases) of DLBCL, and 65% (11 of 17 cases) of MLBCL were negative for c-Jun despite staining of appropriate internal controls (representative DLBCL, Fig. 2H; Table 2). Of note, in the rare Gal1+ DLBCLs and MLBCLs, coexpression of c-Jun was observed (Supplementary Fig. S1; Table 2). More specifically, of the 213 cases immunostained for both Gal1 and c-Jun, only 3 cases (1.4%) showed Gal1 expression in the absence of c-Jun. Taken together, these data reveal a strong association between the presence of Gal1 and the coexpression of nuclear c-Jun, regardless of the lymphoma type.

Association between Gal1 expression and phosphorylated (activated) c-Jun. The transcription factor, c-Jun, is constitutively expressed in Hodgkin cell lines and primary cHL tumors (13), and is a critical component of the AP1 transcriptional complex required for Gal1 expression (13). To confirm that

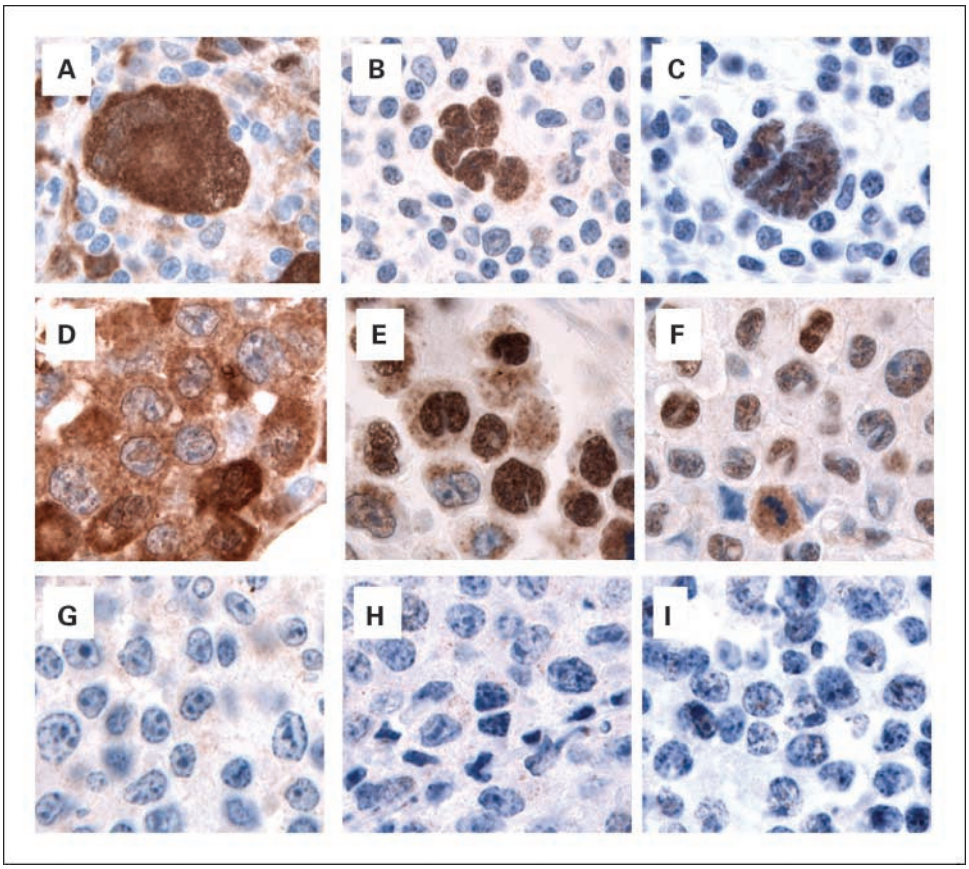

Fig. 2. Matched primary human tumor samples stained for Gal1 (*A, D* and *G*), c-Jun (*B, E*, and *H*), or c-Jun phosphorylated on serine 63 (*C, F*, and *I*) and photographed at ×1,000 magnification. RS cells of cHL with positive staining for Gal1 (*A*), c-Jun (*B*), and phosphorylated c-Jun (*C*). Malignant cells of ALCL with positive staining for Gal1 (*D*), c-Jun (*E*), and phosphorylated c-Jun (*F*). Malignant cells of DLBCL with negative staining for Gal1 (*G*), c-Jun (*H*), and phosphorylated c-Jun (*I*).

Table 2. Coexpression of Galectin-1 and c-Jun in Lymphomas with Large Cell Morphology

Diagnosis	Gal1+ and c-Jun+	Gal1+ and c-Jun-	Gal1- and c-Jun+	Gal1- and c-Jun-
cHL*	54/60 (90%)	0/60 (0%)	6/60 (10%)	0/60 (0%)
NLPHL	0/15 (0%)	0/15 (0%)	2/15 (13%)	13/15 (87%)
MLBCL	2/17 (12%)	0/17 (0%)	4/17 (24%)	11/17 (65%)
DLBCL	4/102 (4%)	3/102 (3%)	14/102 (14%)	81/102 (79%)
ALCL	18/19 (95%)	0/19 (0%)	0/19 (0%)	1/19 (5%)

*Cases of cHL for which both Gal1 and c-Jun staining could be evaluated.

the c-Jun protein detected by immunohistochemistry is functionally active, we did immunostaining on a subset of tumors with a monoclonal antibody that specifically recognizes the activated form of c-Jun (phospho Ser63 c-Jun; ref. 24). In all cases of Gal1+ cHL, there was corresponding expression of the activated phosphorylated form of c-Jun in RS cells (Supplementary Fig. S1; Fig. 2C). Similarly, all examined ALCLs expressed both Gal1 and phospho c-Jun (Fig. 2F). The rare cases of DLBCL and MLBCL that expressed Gal1 also exhibited nuclear c-Jun and phospho c-Jun staining (Supplementary Fig. S1).

Discussion

We previously showed that the immunoregulatory carbohydrate-binding protein, Gal1, is produced by Hodgkin cell lines in a manner dependent on constitutive activation of c-Jun and the AP1 transcription complex (13). Herein, we extend these observations to a large series of primary cHLs and show near-uniform Gal1 staining, and the expression and activation of c-Jun, in RS cells of all cHL subtypes. In contrast, despite certain overlapping morphologic and molecular features, other B-cell lymphomas including NLPHL, MLBCL, and DLBCL are largely negative for Gal1 and c-Jun expression and activation. However, we find that ALCL consistently exhibits Gal1 and c-Jun expression and immunohistochemical evidence of active AP1 signaling. Taken together, these findings define a functional AP1 signature in both cHL and ALCL that includes an AP1 component (c-Jun) and a clinically relevant AP1 target (Gal1).

Our observations have several implications for both the understanding of lymphoma cell biology and the practice of diagnostic pathology. We previously have shown that AP1-dependent production of Gal1 by Hodgkin cell lines promotes an immune privileged microenvironment by favoring the expansion of Th2 cells at the expense of Th1 cells and the retention and/or expression of T regulatory cells (13). These findings provide a mechanistic basis for the relative abundance of Th2 cells and Th2-associated cytokines that accompany malignant RS cells in primary biopsy samples of cHL (25). There are at least two consequences for the survival and expansion of RS cells in cHL. First, it is recognized that RS cells are dependent on the constitutive activation of the signaling molecule STAT6 for survival and growth. STAT6 activation, in turn, is triggered by cell surface receptors that recognize the Th2 associated cytokine, interleukin 13 (26). By skewing the T-cell population toward a Th2 phenotype, Gal1 likely promotes the survival and growth of RS cells. Second, in a recently described murine melanoma model, the expression of Gal1 by malignant cells directly suppressed T-cell–mediated tumor rejection *in vivo* (9). Similarly, abundant Gal1 expression by RS cells may promote a tolerant immunologic environment for

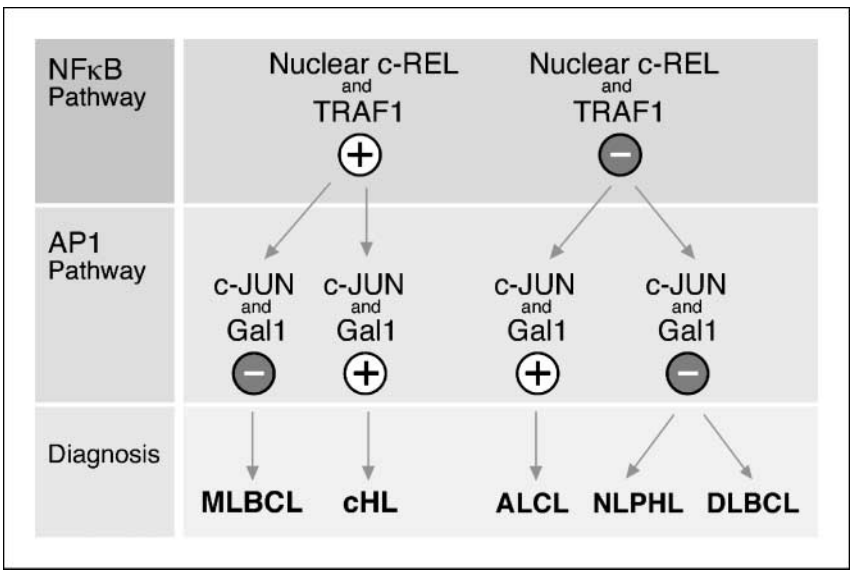

Fig. 3. Algorithm for the immunohistochemical subclassification of primary MLBCL, cHL, ALCL, NLPHL, and DLBCL using and nuclear c-REL and TRAF1, and c-Jun and Gal1. c-REL and TRAF1 are related to the nuclear factor-κB pathway and immunoreactivity aids in the distinction of cHL and MLBCL from other lymphomas. c-Jun and Gal1 are associated with the AP1 pathway and immunoreactivity help separate cHL and ALCL from MLBCL, DLBCL, and NLPHL.

malignant cells despite the magnitude of the inflammatory infiltrate (13). A recent report associating RS Gal1 expression and impaired EBV-specific immune responses is consistent with this hypothesis (22).

We find that 92% of examined primary cHL express high levels of Gal1 within the RS cells. This includes all subtypes of cHL (nodular sclerosis, mixed cellularity, lymphocyte rich and cHL not otherwise specified). The frequency of Gal1 expression in our series exceeds that recently reported by another group (62% of cases of EBV+ cHL; ref. 22). The lower reported incidence of Gal1 expression within the tumor cells in that study is likely due to the commercial Gal1 antibody used, which is less sensitive in detecting moderate levels of Gal1 than the two independent Gal1 antisera from our laboratories. We also find that Gal1 expression is not restricted to EBV+ cHL indicating that the protein likely plays an immunomodulatory role beyond the suppression of EBV-specific cytotoxic T cells.

Intriguingly, we also identified rare cases of cHL that do not express detectable levels of the protein within the RS cells. Review of these cases confirmed typical features of cHL and revealed no obvious morphologic variations in the degree of inflammatory infiltrate associated with the tumor cells. It will be of interest, however, to determine whether these rare tumors differ in the composition of their T-cell infiltrate or in their response to therapy.

Given that Hodgkin RS cell Gal1 expression is dependent on AP1 signaling *in vitro*, we examined c-Jun expression and activation in our primary cHL samples. In agreement with prior reports (23), we found that c-Jun is consistently expressed in the RS cells of cHL. Furthermore, using an antibody recognizing a serine phosphoepitope at amino acid position 63 that is specific for activated c-Jun, we find evidence for active AP1 signaling in every case of cHL. This finding is similar to that of a recent report that used an antibody that recognizes phosphorylated-c-Jun but cross-reacts with phospho-JunD (27).

In contrast to cHL, a distinctly different pattern of Gal1 expression and c-Jun activation was observed in NLPHL. In these tumors, moderate to strong Gal1 expression was not present and c-Jun staining was rarely detected. Interestingly, we found that a subset of cases of NLPHL exhibited weak Gal1 reactivity exclusively in a perinuclear pattern. We interpreted this staining as negative for Gal1 because the subcellular localization and magnitude of Gal1 staining greatly differ from that seen in other Gal1+ tumors.

Despite the recent observation that cHL and certain forms of DLBCL such as MLBCL possess similarities at the molecular level (15, 28), the tumor cells of MLBCL only rarely expresses Gal1 or c-Jun. Further, our examination of nonmediastinal DLBCLs indicates that Gal1 expression and AP1 activation is uncommon in the neoplastic B-cells of these tumors (7% cases). A minority of DLBCLs (18%) expressed c-Jun, and the coexpression of both Gal1 and c-Jun occurred in only 4% of cases of DLBCL. The presence of both Gal1 and c-Jun, therefore, is a discriminating characteristic of these tumor cells and is highly sensitive (92%) and specific (96%) for the diagnosis of cHL versus DLBCL.

Finally, we found that ALCL, an aggressive, large cell lymphoma of T-cell lineage, also expresses high levels of Gal1. In agreement with a prior report (27), we confirmed that c-Jun is expressed by the vast majority of ALCLs, and we found that all c-Jun+ cases also exhibit phospho-c-Jun immunostaining. These findings suggest that in ALCL, AP1 signaling likely drives Gal1 expression. Furthermore, Gal1 expression in ALCL may promote a more permissive microenvironment for tumor survival and growth. Although there are fewer infiltrating nonneoplastic T lymphocytes in ALCL compared with cHL, we did observe variable numbers of small, infiltrating lymphocytes in many of the primary ALCLs (29). With emerging interest in immunotherapy targeting ALK kinase in ALCL (30), understanding the mechanisms limiting effective antitumor responses will be important.

Recently, we showed that constitutive activation of the canonical nuclear factor-κB signaling pathway, as identified by the presence of nuclear c-REL containing heterodimers and increased expression of the nuclear factor-κB target and signaling molecule, TRAF1, is a common feature of both cHL and MLBCL that serves to distinguish these tumors from nonmediastinal DLBCL (17, 31). Here, we report that the majority of MLBCLs resemble nonmediastinal DLBCLs in failing to express Gal1 and c-Jun. Thus, immunostaining for the combination of the above-mentioned markers can be used to aid in distinguishing these entities when conventional morphology and immunophenotyping fail to establish a clear diagnosis (Fig. 3).

In summary, we provide evidence for constitutive AP1-dependent Gal1 expression in the RS cells of cHL and the tumor cells of ALCL. Furthermore, combined c-Jun and Gal1 expression is not observed in NLPHL and is rarely seen in the tumor cells of MLBCL and DLBCL. These findings identify a functional AP1 signature within cHL and ALCL tumor cells and suggest that inhibition of Gal1 expression or neutralization of Gal1 protein could be a potential means of augmenting an antitumor response and improving treatment of these tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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