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PAX5-positive T-cell anaplastic large cell lymphomas associated with extra copies of the *PAX5* gene locus

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Cell lineage is the major criterion by which lymphomas are classified. Immunohistochemistry has greatly facilitated lymphoma diagnosis by detecting expression of lineage-associated antigens. However, loss or aberrant expression of these antigens may present diagnostic challenges. Anaplastic large cell lymphoma is a T-cell lymphoma that shows morphologic and phenotypic overlap with classical Hodgkin's lymphoma, which is a tumor of B-cell derivation. Staining for the B-cell transcription factor, paired box 5 (PAX5), has been suggested to be helpful in this differential, as it is positive in most classical Hodgkin's lymphomas, but absent in anaplastic large cell lymphomas. In this study we report four systemic T-cell anaplastic large cell lymphomas that were positive for PAX5 by immunohistochemistry, with weak staining intensity similar to that observed in classical Hodgkin's lymphoma. All diagnoses were confirmed by a combination of morphologic, phenotypic, and molecular criteria. Three cases were anaplastic lymphoma kinase (ALK) negative and one was ALK positive. PAX5 immunohistochemistry was negative in 198 additional peripheral T-cell lymphomas, including 66 anaplastic large cell lymphomas. Unexpectedly, although *PAX5* translocations were absent, all evaluable PAX5-positive anaplastic large cell lymphomas showed extra copies of the *PAX5* gene locus by fluorescence *in situ* hybridization (FISH). In contrast, only 4% of PAX5-negative peripheral T-cell lymphomas had extra copies of *PAX5*. We conclude that aberrant expression of PAX5 occurs rarely in T-cell anaplastic large cell lymphomas, and may be associated with extra copies of the *PAX5* gene. PAX5-positive lymphomas with morphologic features overlapping different lymphoma types should be evaluated with an extensive immunohistochemical panel and/or molecular studies to avoid diagnostic errors that could lead to inappropriate treatment. As PAX5 overexpression causes T-cell neoplasms in experimental models, PAX5 may have contributed to lymphomagenesis in our cases.
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Cell lineage is the major criterion by which lymphomas are classified.[1] In routine clinical practice, the B- or T-cell origin of lymphomas is determined using immunophenotyping studies to detect lineage-associated antigens expressed by the tumor cells. Occasionally, however, loss or aberrant expression of lineage-associated antigens may present diagnostic challenges. One such challenge is the differential diagnosis between T-cell anaplastic large cell lymphoma and classical Hodgkin's lymphoma, which is a tumor of B-cell derivation.

Anaplastic large cell lymphoma and classical Hodgkin's lymphoma can show considerable morphologic overlap.[2,3] Anaplastic large cell lymphomas and other peripheral T-cell lymphomas may have Reed–Sternberg-like cells and a prominent mixed inflammatory background, leading to the introduction of the term, 'Hodgkin-like' anaplastic large cell lymphoma.[4,5] Conversely, some cases of classical Hodgkin's lymphoma are rich in tumor cells and have a minimal inflammatory background,

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resembling anaplastic large cell lymphoma.[6] In fact, many of the tumors originally considered 'Hodgkin-like' anaplastic large cell lymphomas subsequently were reclassified as classical Hodgkin's lymphomas.[7,8]

In addition to their morphologic features, anaplastic large cell lymphoma and classical Hodgkin's lymphoma may show striking phenotypic overlap. Classical Hodgkin's lymphomas typically express CD30 and CD15, lack expression of multiple B-cell antigens, and may aberrantly coexpress T-cell antigens and cytotoxic proteins.[9,10] Anaplastic large cell lymphomas and some peripheral T-cell lymphomas express CD30, may coexpress CD15,[11–14] and often lack expression of multiple T-cell antigens despite having clonal T-cell receptor (TCR) gene rearrangements.[15,16] In addition, occasional peripheral T-cell lymphomas aberrantly express B-lineage markers such as CD20 and CD79a.[17–21] When present, expression of anaplastic lymphoma kinase (ALK) as a result of *ALK* gene translocation is helpful in establishing the diagnosis of anaplastic large cell lymphoma rather than classical Hodgkin's lymphoma.[22] However, approximately 45% of anaplastic large cell lymphomas are ALK negative.[23,24] Correct diagnosis is critical, as classical Hodgkin's lymphoma and ALK-negative anaplastic large cell lymphoma are treated differently, and are associated with an 85% cure rate in the former and $<50\%$ 5-year overall survival in the latter.[24,25]

The paired box 5 (PAX5) transcription factor (B-cell-specific activating protein (BSAP)) is necessary for B-lineage commitment,[26–28] and has shown excellent specificity for B-cell lineage by immunohistochemistry.[29–33] PAX5 staining may be helpful in the differential diagnosis between classical Hodgkin's lymphoma and ALK-negative anaplastic large cell lymphoma, as it shows characteristic weak staining in most classical Hodgkin's lymphomas[34] and 'should be negative in all cases of anaplastic large cell lymphoma', according to the 2008 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues.[8]

The purpose of this study was to characterize the morphologic, phenotypic, and genetic features of four cases of PAX5-positive anaplastic large cell lymphomas observed in our practice, and to compare these features with 198 additional peripheral T-cell lymphomas. Our findings indicate that PAX5 can be observed in otherwise typical anaplastic large cell lymphomas, and thus cannot be solely relied upon to distinguish anaplastic large cell lymphoma from classical Hodgkin's lymphoma. Interestingly, PAX5-positive anaplastic large cell lymphomas showed extra copies of the *PAX5* gene locus, suggesting a possible mechanism for the PAX5 expression, and perhaps contributing to lymphomagenesis in these cases. Our findings support the use of a broad panel of B- and T-cell antigens in assigning lymphoma lineage, with additional molecular studies performed in ambiguous cases.

Materials and methods

During the period 2007 to 2009, four PAX5-positive anaplastic large cell lymphomas were identified from the hematopathology practice at Mayo Clinic, Rochester, Minnesota; 198 additional peripheral T-cell lymphomas from the years 1987 to 2009 identified from the Mayo Clinic archives were studied. All cases were classified based on 2008 WHO criteria.[1]

PAX5 immunohistochemistry was performed on paraffin-embedded tissue sections by pretreating in 1 mM EDTA buffer at pH 8.0 for 30 min at 97 °C (PT Module; Lab Vision, Fremont, CA, USA) and staining for PAX5 (1:200, clone 24, BD Bioscience) on a Dako (Carpinteria, CA, USA) autostainer using the Advance detection system (Dako) with diaminobenzidine as the chromogen. Immunohistochemistry for other markers was performed as previously described[35] using antibodies shown in Table 1. Aside from CD30 and PAX5 (discussed below), immunostaining was scored as strong or weak, and designated as negative (−, no staining), focal (−/+, $<10\%$ of tumor cells), partial (+/−, 10–30% of tumor cells), or positive (+, $>30\%$ of tumor cells).

Polymerase chain reaction (PCR) for TCR γ-chain and immunoglobulin gene rearrangements was performed as described previously.[36,37] Fluorescence *in situ* hybridization (FISH) for *PAX5* was performed and scored as described previously using a home-brew break-apart probe.[38] In brief, DNA was isolated from bacterial artificial chromosome probes (ResGen Invitrogen; Carlsbad, CA, USA) spanning the *PAX5* locus as shown in Figure 3c. Probes were labeled with SpectrumOrange-dUTP or SpectrumGreen-dUTP using nick translation (Abbott Molecular, Des Plaines, IL, USA) and hybridized to tissue sections. Cases with ≥ 4 fusion signals were considered to have extra copies of the *PAX5* gene locus.

Additional peripheral T-cell lymphomas were evaluated using immunohistochemistry and/or FISH as indicated above on tissue microarrays constructed from paraffin blocks as previously described.[39] The study was approved by the institutional review board and biospecimens committee of Mayo Clinic.

Results

Clinicopathologic Findings of PAX5-Positive Anaplastic Large Cell Lymphomas

The clinicopathologic features of the four PAX5-positive anaplastic large cell lymphomas are summarized in Table 2. There were two males and two females with an age range of 31 to 87 years. Three patients (cases 1–3) presented with lymphadenopathy and one (case 4) presented with a pathologic fracture of L4; imaging revealed masses in the neck, chest, and abdomen. Treatment data are available for three patients. One (case 1) had severe cardiac

Table 1 Antibodies used in immunophenotypic analyses

Antigen	*Clone*	*Dilution*	*Source*
ALK	ALK1	1:100	Dako (Carpinteria, CA, USA)
BetaF1	8A3	1:100	Endogen (Woburn, MA, USA)
BOB1	TG14	1:200	Novocastra (Newcastle upon Tyne, UK)
CD2	AB75	1:100	Novocastra
CD3	PS1	1:50	Novocastra
CD4	4B12	1:600	Novocastra
CD5	4C7	1:300	Novocastra
CD7	LP15	1:200	Novocastra
CD8	C8/144B	1:100	Dako
CD15	MMA	1:50	BD Biosciences (Franklin Lakes, NJ, USA)
CD19	LE-CD19	1:200	Dako
CD20	L26	1:200	Dako
CD22	FPC1	1:200	Novocastra
CD30	Ber-H2	1:20	Dako
CD43	L60	1:10 000	BD Biosciences
CD45	2B11+ PD7/26	1:1500	Dako
CD56	123C3	1:25	Monosan/Caltag (Burlingame, CA, USA)
CD79a	JCB117	1:50	Dako
Clusterin	41D	1:200	Upstate (Lake Placid, NY, USA)
EMA	E29	1:50	Dako
Granzyme B	GRB-7	1:100	Monosan/Caltag
OCT2	OCT-207	1:100	Novocastra
PAX5	24	1:200	BD Biosciences
TIA-1	TIA-1	1:200	Immunotech (Fullerton, CA, USA)

disease precluding systemic chemotherapy. He was treated with palliative radiotherapy for edema caused by bulky inguinal and pelvic adenopathy, and died 2 months later. Two patients (cases 2 and 4) were treated with cyclophosphamide, doxorubicin hydrochloride, oncovin, and prednisone (CHOP), and achieved a partial response at 6 months (4 cycles) and a complete response at 3 months (3 cycles), respectively.

Morphologic features in all four cases were characteristic of anaplastic large cell lymphoma (Figures 1 and 2). All showed sheets of medium-sized to large lymphocytes with variably folded or horseshoe-shaped nuclei typical of so-called ‘hallmark’ cells.[40] Reed–Sternberg cells were absent. A sinusoidal pattern of distribution was observed in cases with lymph node material available, most prominently noted in case 2 (Figure 1e). Occasional inflammatory cells were present in the background, particularly in case 3 (Figure 2a).

All cases showed uniform, strong staining for CD30 by immunohistochemistry (Figures 1b and f and 2b and f). One case (case 4) was positive for ALK (predominantly cytoplasmic; Figures 2g). All cases were negative for CD3 and showed variable positivity for other T-cell antigens; of these, CD2 and CD4 were most commonly observed, with at least focal staining observed in cases 3 and 4, respectively (Figures 1c and g). Cytotoxic marker expression (TIA-1 or granzyme B) was observed at least focally in three cases. CD15 expression was observed in one case (case 3). Expression of EMA and clusterin was observed in two cases. PAX5 positivity was observed in >80% of tumor cells in all cases, was solely nuclear, and was weaker than that observed in reactive B cells (Figures 1d and 2d), similar to the typical staining intensity of Reed–Sternberg cells in classical Hodgkin’s lymphoma. Other surface B-lineage markers (CD19, CD20, CD22, and CD79a) were negative. OCT2 (POU2F2) and BOB1 (POU2AF1 or OBF1) were at least focally positive in two cases and one case, respectively.

Table 2 Clinical features and results of immunohistochemistry and molecular studies in PAX5-positive anaplastic large cell lymphomas

	Case 1	*Case 2*	*Case 3*	*Case 4*
Clinical features				
Age (year)/gender	87/M	31/F	45/F	53/M
Biopsy site	Inguinal lymph node	Axillary lymph node	Axillary lymph node	L4 vertebra
Stage	III	IIIA	Unknown	IVB
Outcome	Died of disease	Alive, PR	Unknown	Alive, CR
Follow-up (months)	2	6	–	3
Immunohistochemistry[a]				
ALK	–	–	–	+
BetaF1	–	ND	–	–
BOB1	–	–	–/+ (w)	–
CD2	+	–/+	+/–	–
CD3	–	–	–	–
CD4	+	+	+/–	+/– (w)
CD5	–	–	+	–
CD7	–	–/+	ND	–
CD8	–	–	ND	–
CD15	–	ND	+	–
CD19	–	–	–	–
CD20	–	–	–	–
CD22	–	–	–	–
CD30	+	+	+	+
CD43	+	ND	+/–	–
CD45	+ (w)	–	+/–	+ (w)
CD79a	–	–	–	–
Clusterin	–	–/+ (w)	–	+
EMA	–	+/– (w)	–	+
Granzyme B	–	ND	–/+	–/+
OCT2	–	–	+ (w)	–/+ (w)
PAX5	+ (w)	+ (w)	+ (w)	+ (w)
TIA-1	–	–/+	–/+	–
Gene rearrangement (PCR)				
T-cell receptor	Positive	Negative	Positive	Failed
Immunoglobulin	Negative	Negative	Negative	Failed
FISH				
≥4 copies of *PAX5*	Yes	Yes	Yes	Failed

PR, partial response; CR, complete response; ND, not done; (w), weak; –, negative; –/+, <10% of tumor cells; +/–, 10–30%; +, >30%.
[a]Immunohistochemical staining intensity was strong unless otherwise indicated.

All cases were evaluated by PCR for clonal TCR and immunoglobulin gene rearrangements. PCR failed in case 4 (decalcified specimen). Two of the remaining three cases showed clonal TCR gene rearrangements (Figures 3a and b). None showed a

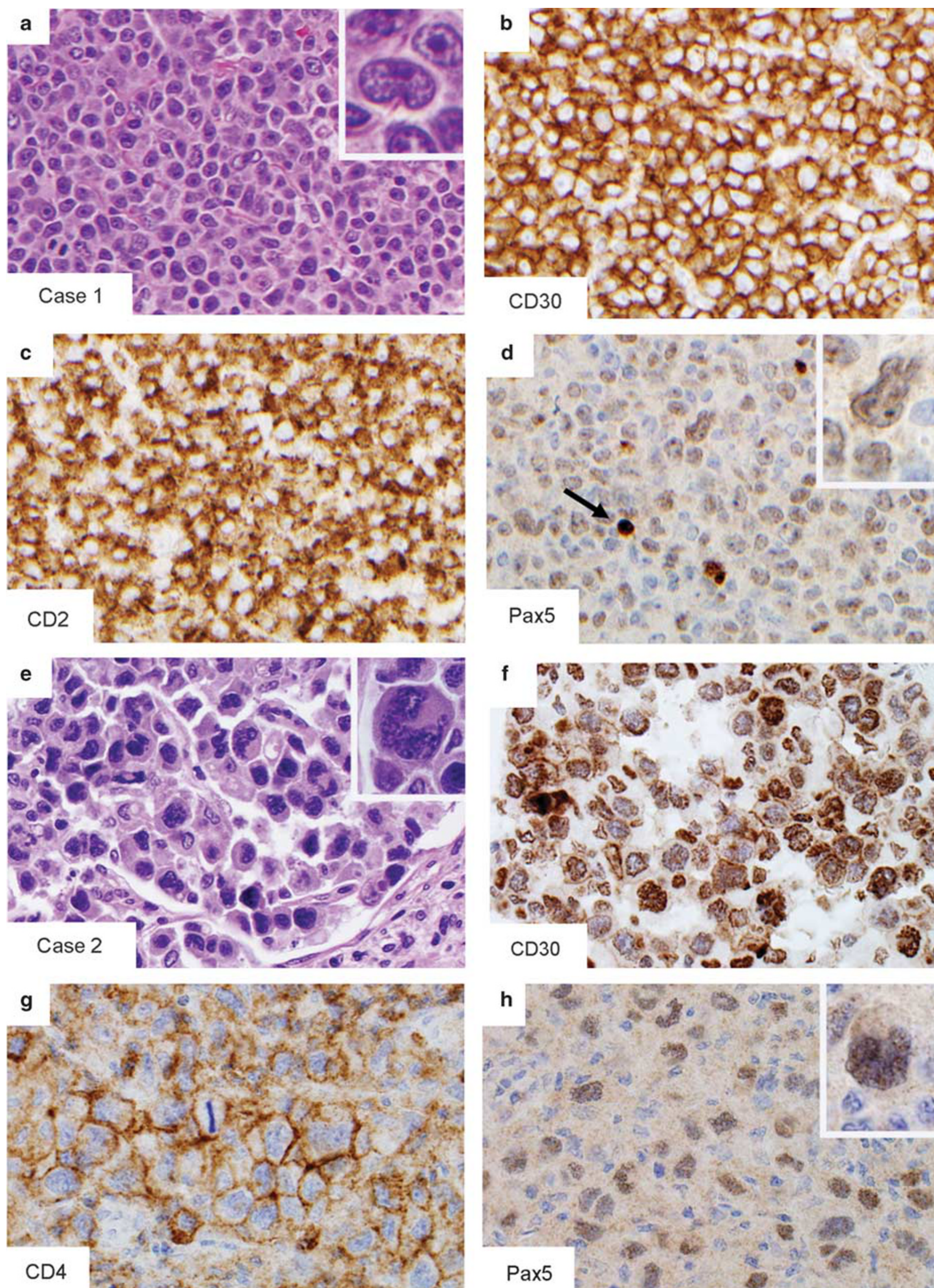

Figure 1 Histological and immunophenotypic features of PAX5-positive anaplastic large cell lymphomas (original magnification ×400; insets, ×1000). (**a–d**) Case 1: ALK-negative anaplastic large cell lymphoma. Hematoxylin and eosin (H&E)-stained slides of a lymph node (**a**) shows sheets of hallmark cells without a significant inflammatory background. The tumor cells are positive for CD30 (**b**) and CD2 (**c**). PAX5 (**d**) shows weak nuclear positivity in the large tumor cells, compared with strong positivity in occasional small B cells (arrow). (**e–h**) Case 2: ALK-negative anaplastic large cell lymphoma. H&E-stained slides of a lymph node show hallmark cells within sinuses (**e**). The tumor cells are positive for CD30 (**f**) and CD4 (**g**), and are weakly positive for PAX5 (**h**).

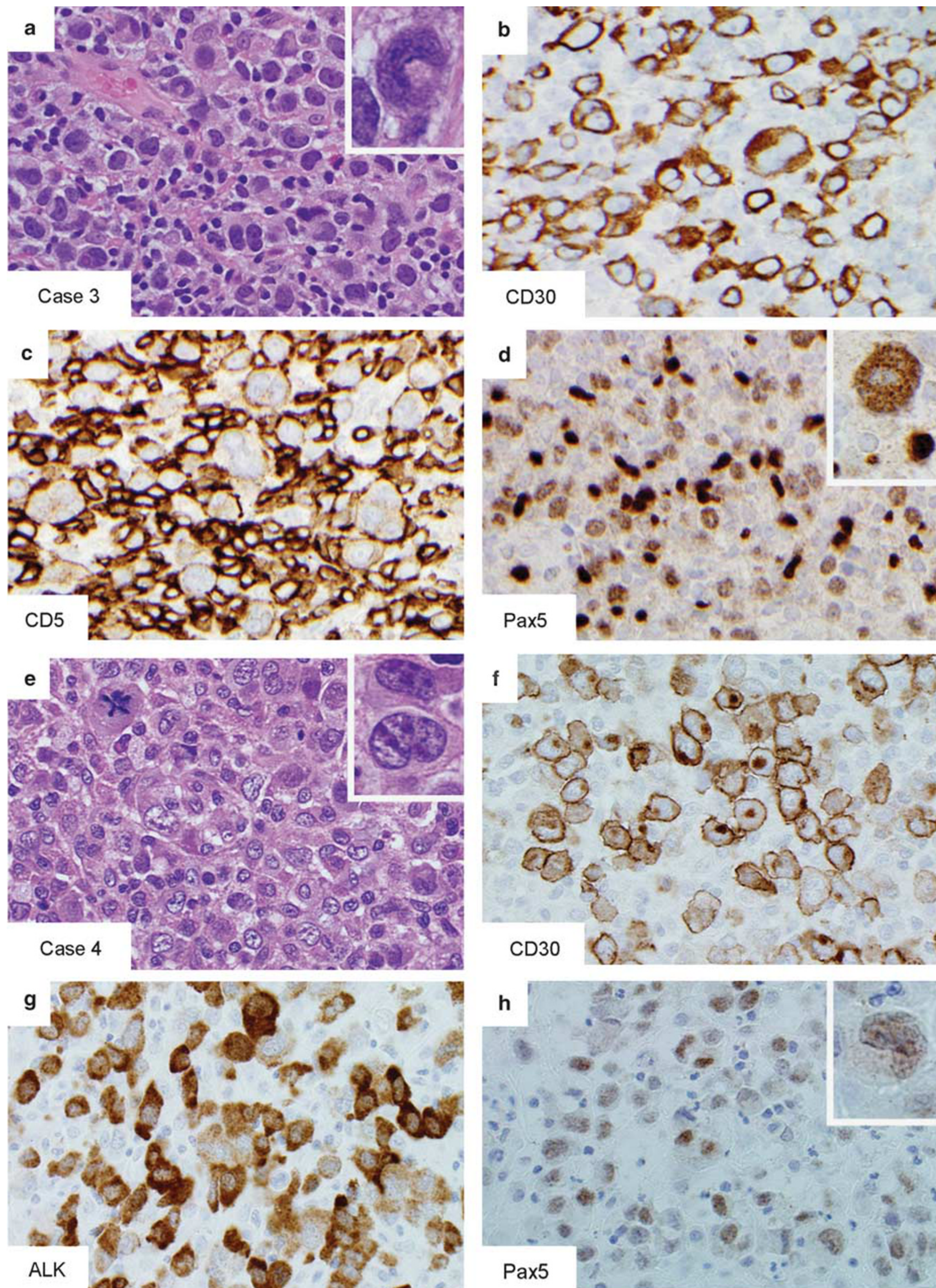

Figure 2 Histological and immunophenotypic features of PAX5-positive anaplastic large cell lymphomas, *continued* (original magnification ×400; insets, ×1000). (**a–d**) Case 3: ALK-negative anaplastic large cell lymphoma. H&E-stained slides of a lymph node show numerous hallmark cells (**a**). The tumor cells are positive for CD30 (**b**) and CD5 (**c**). PAX5 (**d**) is more weakly positive in the tumor cells (inset, upper left) than in admixed small B cells (inset, lower right). (**e–h**) Case 4: ALK-positive anaplastic large cell lymphoma. H&E-stained slides of an L4 vertebral mass show numerous hallmark cells (**e**). The tumor cells are positive for CD30 (**f**) and ALK (**g**), and are weakly positive for PAX5 (**h**).

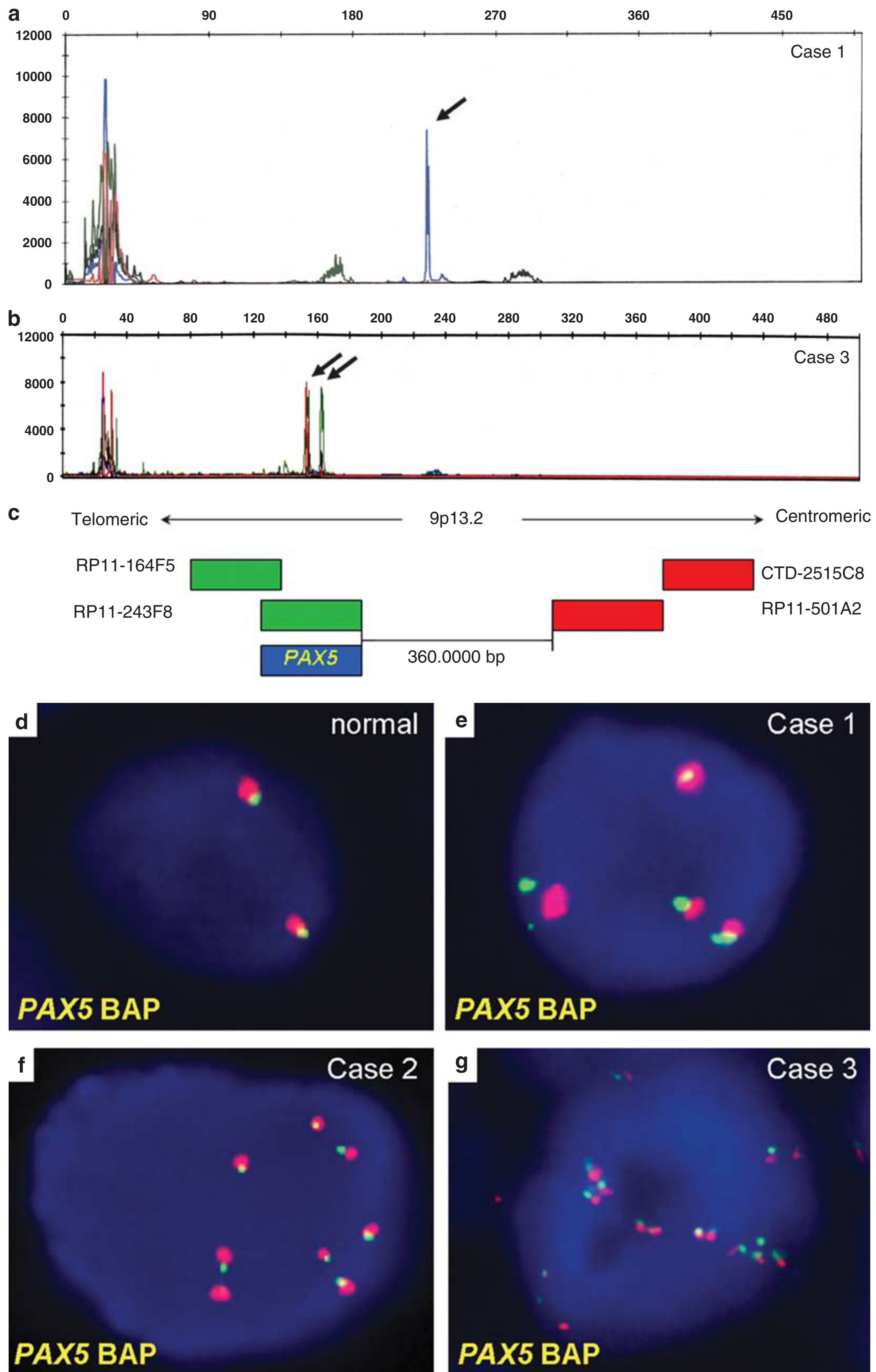

Figure 3 Molecular features of PAX5-positive anaplastic large cell lymphomas. (**a**,**b**) PCR for T-cell receptor γ-chain gene rearrangement in cases 1 (**a**) and 3 (**b**) show clonal peaks (arrows). (**c**) FISH was performed using a break-apart probe for the *PAX5* gene locus on 9p13.2, with bacterial artificial chromosome (BAC) designations as shown. Centromeric and telomeric BACs were labeled red and green, respectively. Relative location of *PAX5* is shown in blue. (**d**) A normal cell shows two fusion signals by FISH. (**e–g**) Cells from PAX5-positive anaplastic large cell lymphomas show extra copies of the *PAX5* gene locus.

clonal immunoglobulin gene rearrangement. Karyotyping was not performed. FISH for the *PAX5* gene locus was performed in all cases. Hybridization failed in case 4. Extra copies of *PAX5* were observed in all remaining cases, with copy numbers ranging from 4 in case 1 to >10 in case 3 (Figures 3e–g). No *PAX5* translocation was found.

Immunohistochemical and FISH Studies of Additional T-Cell Lymphomas

PAX5 was evaluated using immunohistochemistry in 198 additional patients (117 males and 81 females; mean age, 59 years) with the following peripheral T-cell lymphoma subtypes: 25 angioimmunoblastic T-cell lymphomas; 66 anaplastic large cell lymphomas (22 ALK positive, 33 ALK negative, and 11 cutaneous); 82 peripheral T-cell lymphomas, NOS; 10 extranodal NK/T-cell lymphomas, nasal type; 6 cases of mycosis fungoides; 2 subcutaneous panniculitis-like T-cell lymphomas; 2 hepatosplenic T-cell lymphomas; 2 enteropathy-associated T-cell lymphomas; 2 T-cell large granular lymphocytic leukemias; and 1 T-cell prolymphocytic leukemia. All were negative for PAX5. Of these, 109 cases were evaluated by FISH for *PAX5*, and 92 showed hybridization adequate for interpretation. No *PAX5* translocation was found. Of the 92 PAX5 protein-negative peripheral T-cell lymphomas, four (4%) had extra copies of the *PAX5* gene locus. All were peripheral T-cell lymphomas, NOS. None resembled anaplastic large cell lymphoma morphologically. CD30 was negative in three and partially positive in one case (10–30% of tumor cells). Other B-cell markers were negative.

Discussion

We report four cases of PAX5-positive T-cell anaplastic large cell lymphoma. Extra copies of the *PAX5* gene locus were shown in all three cases evaluable by FISH. PAX5 is a transcription factor in the paired-box-containing family, which is involved in control of organ development and tissue differentiation.[41] PAX5 has an essential role in B-lymphoid lineage commitment,[26–28] and is widely used as a B-cell marker in immunohistochemical evaluation of lymphoid tissues.[30] Anaplastic large cell lymphomas may share morphologic and phenotypic features with B-lineage neoplasms, particularly classical Hodgkin's lymphoma. Therefore, our findings have important implications for interpreting PAX5 immunohistochemistry in lymphoma classification.

Our PAX5-positive anaplastic large cell lymphomas had clinical presentations, histological features, and phenotypes (other than PAX5 expression) that are characteristic of anaplastic large cell lymphoma, allowing definitive classification despite the unusual positivity for PAX5. Consistent with previously published data,[42] the three ALK-negative cases lacked clonal immunoglobulin gene rearrangements, and two of three had clonal TCR gene rearrangements. Case 3 showed coexpression of CD15, a finding typical of classical Hodgkin's lymphoma but that may also be observed in anaplastic large cell lymphoma.[12–14] The other features did not support a diagnosis of classical Hodgkin's lymphoma. There were characteristic hallmark cells with only occasional inflammatory cells observed in the background. In addition to the expression of T-cell antigens and cytotoxic markers, the tumor cells expressed BOB1 and (focally) OCT2, which are the transcription factors typically absent in classical Hodgkin's lymphoma.[43] Finally, the presence of a clonal TCR gene rearrangement and absence of clonal immunoglobulin gene rearrangement support the diagnosis of anaplastic large cell lymphoma in this case. Case 4 was a decalcified specimen and molecular studies were unsuccessful, but positivity for ALK assisted in confirming the diagnosis of anaplastic large cell lymphoma.

In a study of cases with overlapping features of anaplastic large cell lymphoma and classical Hodgkin's lymphoma, Tamaru *et al*[44] found weak PAX5 expression in 3 of 17 ALK-negative anaplastic large cell lymphomas and 0 of 11 ALK-positive anaplastic large cell lymphomas. Although gene rearrangement studies were not performed to confirm T-cell origin, the three PAX5-positive tumors expressed both CD45 and BOB1, and two expressed EMA. These immunophenotypic features support the diagnosis of ALK-negative anaplastic large cell lymphoma rather than classical Hodgkin's lymphoma. The tumors lacked T-cell antigen expression, except for CD45RO in one case and TIA-1 in another, and were negative for OCT2. The phenotypes of our cases were similar in the intensity of PAX5 staining and variable staining for EMA. We found more consistent positivity for T-cell antigens and observed OCT2 expression in two cases; conversely, BOB1 was observed focally in only one of our cases and CD45 expression was more variable. In addition, one of our cases was ALK positive.

A single previous case of peripheral T-cell lymphoma NOS, expressing PAX5 was reported by Tzankov *et al*.[45] No PAX5-positive cases were identified in additional peripheral T-cell lymphomas studied by Tzankov *et al* ($n=43$),[45] Krenacs *et al* ($n=20$),[31] Foss, *et al* ($n=40$),[34] or Torlakovic *et al* ($n=26$).[32] We did not identify any additional PAX5-positive cases in 198 peripheral T-cell lymphomas, including 66 additional anaplastic large cell lymphomas. Thus, the overall incidence of PAX5 positivity in peripheral T-cell lymphomas appears low. Nevertheless, PAX5 expression is not entirely specific for B-cell lineage in lymphomas. Furthermore, occasional non-lymphoid neoplasms express PAX5, including t(8;21)-positive acute myelogenous leukemias, small cell carcinomas, and other neuroendocrine tumors.[30]

Translocations between *PAX5* and the immunoglobulin heavy chain gene (*IGH@*) drive PAX5 expression in mature B-cell lymphomas,[46,47] In addition, PAX5 is oncogenic in T cells, as a reconstructed *PAX5*/*IGH@* translocation induces T-cell lymphoblastic lymphomas in mice.[48] Therefore, to analyze the mechanism for PAX5 expression in anaplastic large cell lymphoma, we performed FISH using a *PAX5* break-apart probe. We did not identify *PAX5* translocations. Unexpectedly, however, all (100%) PAX5-positive anaplastic large cell lymphomas with informative FISH studies had extra copies of the *PAX5* gene locus. In contrast, only 4% of PAX5-negative T-cell lymphomas had extra copies of *PAX5*. No PAX5-negative anaplastic large cell lymphoma had extra copies of *PAX5*, and previous genomic studies of anaplastic large cell lymphoma have not identified recurrent gains of 9p, on which *PAX5* resides.[49–51] These findings suggest a possible association between extra copies of *PAX5* and PAX5 protein expression in anaplastic large cell lymphomas. The finding of rare PAX5-negative T-cell lymphomas with extra copies of *PAX5* (all peripheral T-cell lymphomas, NOS) indicates that factors besides gene dosage influence PAX5 protein expression in T-cell lymphomas. *PAX5* methylation is associated with PAX5 negativity in human tumors[52,53] and might represent a mechanism by which T-cell lymphomas with extra copies of *PAX5* do not express PAX5 protein. However, we did not have adequate material to assess gene methylation in our cases.

In conclusion, recognizing the existence of PAX5-positive anaplastic large cell lymphomas is important to avoid incorrectly assigning B-cell lineage to these rare tumors. Specifically, PAX5 cannot always differentiate anaplastic large cell lymphoma from classical Hodgkin's lymphoma, particularly as the intensity of staining in PAX5-positive anaplastic large cell lymphomas is similar to that typically observed in classical Hodgkin's lymphoma. Diagnostic errors can be avoided by interpreting PAX5 immunohistochemistry in the context of clinical features, morphology (including both cytologic features of the tumor cells and cellular background), and a panel of B- and T-lineage-associated antibodies. Molecular studies are recommended in cases with ambiguous lineage. Extra copies of the *PAX5* gene may contribute to PAX5 expression in anaplastic large cell lymphomas. Finally, as PAX5 is oncogenic in T cells,[48] PAX5 expression may have contributed to lymphomagenesis in our cases.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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