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ORIGINAL ARTICLE

Transformation of B cell lymphoma to histiocytic sarcoma: somatic mutations of PAX-5 gene with loss of expression cannot explain transdifferentiation

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Abstract Transdifferentiation of B cell lymphoma of germinal center cell origin to histiocytic sarcoma has recently been described but is a rare occurrence. The cause for loss of B cell differentiation in these lymphomas is unknown. We investigated whether somatic hypermutation of the *PAX-5* gene, a transcription factor that is important for maintaining B cell identity and is frequently mutated in B cell lymphomas of germinal center cell origin, might be a cause for loss of PAX-5 expression and thus B cell phenotype. However, no somatic hypermutation of the *PAX-5* gene was detected in the two cases we studied. The molecular basis for transdifferentiation of B cell lymphoma to histiocytic sarcoma remains therefore unresolved.

Keywords PAX-5 · B-cell · Lymphoma · Histiocytic sarcoma · Transdifferentiation

Introduction

Phagocytes are derived from monocytes that enter the blood and migrate into tissues to differentiate into macrophages/histiocytes [1, 2]. Tumors of histiocytes and dendritic cells are extremely rare and their classification is still controversial [1, 2]. In the last World Health Organization classification [1], primary histiocytic sarcoma (HS) is defined as a tumor with morphologic and immunohistochemical features similar to those of mature histiocytes and usually lack clonal immunoglobulin and T cell receptor genes. Of interest, those tumors frequently occur in patients with another malignant tumor. For example, a subset of cases with HS occurs in patients with previously diagnosed mediastinal germ cell tumors; other cases are associated with malignant lymphomas [3–6].

Of interest is one recent publication which described rare cases of HS occurring as a transformed tumor from B cell lymphoma [7]. Here, we report two such cases and set out to look for a mechanism explaining this transdifferentiation.

Somatic hypermutation affects rearranged immunoglobulin genes in B cells and results in higher affinity of the antibody. However, aberrant somatic hypermutation that affects other genes, including the *PAX-5* gene, has been described in B cell lymphomas [8]. PAX-5 is a transcription factor that is important for maintaining B cell identity [9]. We therefore investigated whether *PAX-5* aberrant somatic hypermutation was a cause of the lack of PAX-5 expression and a probable cause of transdifferentiation.

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Materials and methods

Clinical histories

Patient 1 A 53-year-old male patient was diagnosed in 1995 with stage IVA follicular lymphoma, grade 2 with generalized lymph node and bone marrow involvement. He was treated with chlorambucil/prednisolone for 6 months. A first relapse was detected 7 years later and the patient was treated again.

Table 1 List of the antibodies used for immunohistochemistry

Antibody (clone)	Source	Dilution	HIER
CD45 (LCA, 2B11/PD7/26)	DAKO Cytomation, Denmark A/S	1:100	LpH
CD20 (L-26)	DAKO Cytomation, Denmark A/S	1:300	LpH
CD3 (SP7, rabbit monoclonal)	LabVision Corporation, UK	1:150	LpH
CD79a (SP-18, rabbit monoclonal)	LabVision Corporation, UK	1:400	T/E
PAX-5 (24)	BD Transduction Laboratories™	1:40	T/E
CD10 (56C6)	Novocastra™	1:40	T/E
BCL-6 (PG-B6p)	DAKO Cytomation, Denmark A/S	1:40	T/E
BCL-2 (Bcl-2/100/D5)	Novocastra™	1:20	T/E
CD21 (2G9)	Novocastra™	1:20	T/E
CD68 (PG-M1)	DAKO Cytomation, Denmark A/S	1:250	T/E
CD14 (7)	Novocastra™	1:160	T/E
CD1a (MTB1)	Novocastra™	1:5	T/E
PU1 (polyclonal)	Santa Cruz Biotechnology	1:300	T/E
MAF-B (goat polyclonal)	Santa Cruz Biotechnology	1:2000	T/E
S-100 (polyclonal)	DAKO Cytomation, Denmark A/S	1:1500	LpH
Ki67 (MIB-1)	DAKO Cytomation, Denmark A/S	1:150	T/E

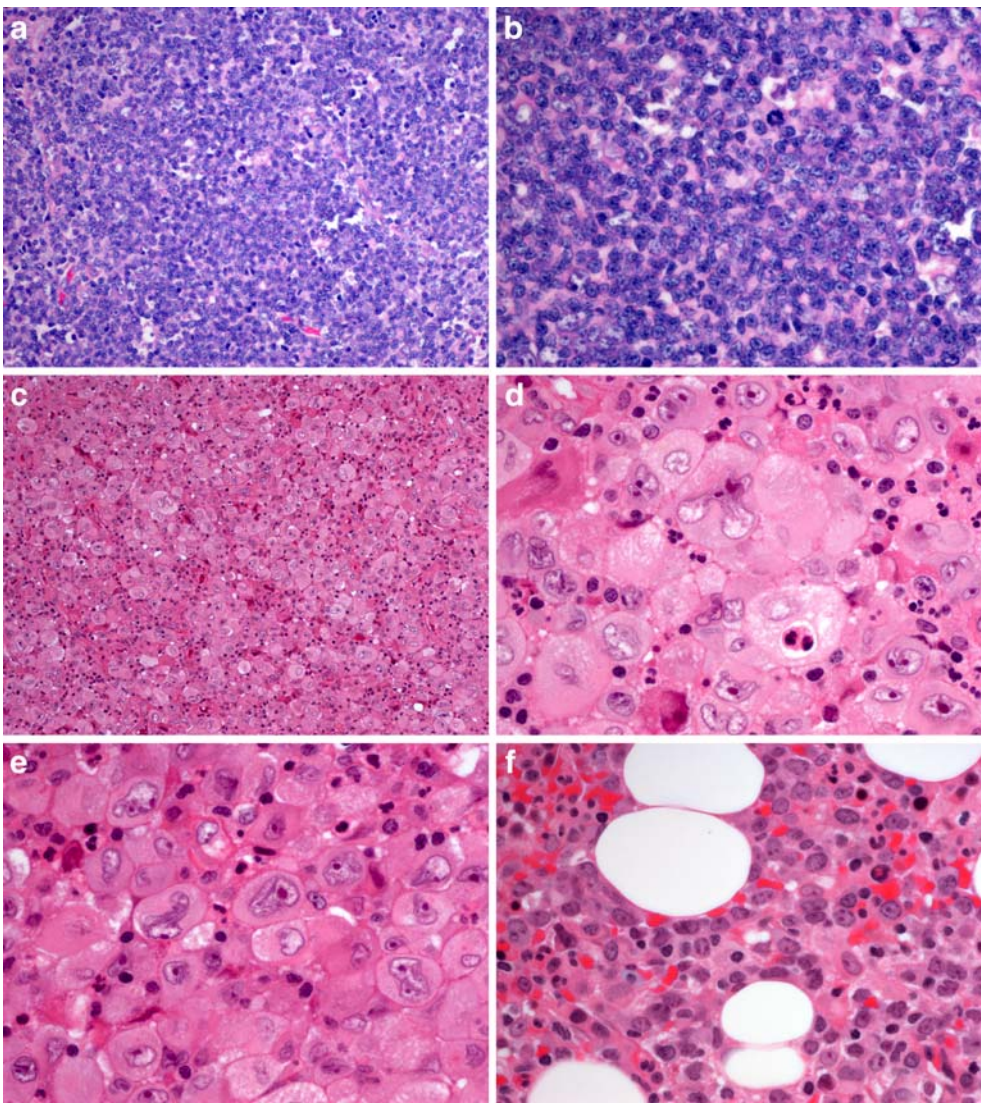

Fig. 1 **a**, **b** Lymph node with follicular lymphoma; **c**, **d**, **e** neck lesion with histiocytic sarcoma; **f** bone marrow infiltration with diffuse large B cell lymphoma (patient 2; H&E)

Table 2 Summarized results for patient 1 and patient 2

	Patient 1				Patient 2		
	Inguinal lymph node, 2001	Inguinal lymph node, 2003	Neck tumor, 2008	Bone marrow, 2008	Tongue, 2006	Tongue, 2007	Skin, 2007
Morphology	Follicular lymphoma, grade 2	Follicular lymphoma, grade 3	Large atypical cells with large vesiculose nuclei and eosinophilic cytoplasm	Diffuse, intraparenchymatous infiltration by centroblasts	Diffuse large B cell lymphoma	Large atypical cells with large vesiculose nuclei and eosinophilic cytoplasm	Large atypical cells with large vesiculose nuclei and eosinophilic cytoplasm
IHC	CD20+, CD79a+, PAX-5+, CD10+, BCL-6+, BCL-2+, PU1+, CD68−, CD14−, CD1a−, S100−, MAF-B-	CD20+, CD79a+, PAX-5+, CD10+, BCL-6+, BCL-2+, PU1+, CD68−, CD14−, CD1a−, S100-, MAF-B-	CD20−, CD79a−, PAX-5−, CD10−/+, BCL-6+, BCL-2−, PU1+, MAF-B+, CD68+, CD14+, CD1a−/+, S100+	CD20+, CD79a+, PAX-5+, CD10+, BCL-6+, BCL-2+, PU1+,CD68−, CD14−, CD1a−, S100−, MAF-B-	CD20+,CD79a, PAX-5+, CD10+, BCL-6+, BCL-2−, PU1+, CD68−, CD14−, CD1a−, S100−,MAF-B−	CD20−, CD79a−, PAX-5−, CD10−, BCL−6+, BCL-2−, PU1+, CD68+, CD14+/−, CD1a−/+, S100+, MAF-B−	CD20−, PAX-5−, CD10−/+, BCL-6+, BCL-2−, PU1+, CD68+, CD14−/+, CD1a−/+,S100+, MAF-B−
FISH	t(14;18)(18q21)	t(14;18)(18q21)	t(14;18)(18q21)	t(14;18)(18q21)	t(14;18)(18q21)	t(14;18)(18q21)	t(14;18)(18q21)
PCR analysis	Not performed	Monoclonal IgH gene rearrangement, one peak 326/327 bp	Monoclonal IgH gene rearrangement, one peak 326/327 bp	Not performed	Monoclonal IgH gene rearrangement, one peak 118 bp	Not performed	Monoclonal IgH gene rearrangement, one peak 118 bp
Sequencing	Intact structure of the gene	Intact structure of the gene	Intact structure of the gene	Not performed	Not performed	Intact structure of the gene	Not performed
EM	Not performed	Not performed	Birbeck granules	Not performed			
Cytogenetic data	48,XY, +2,add(13)(q33), +8,t(14;18)(q32;q21), +17[14]/46,XY[1]	43-49,XY, +2,del(6)(q14), +8,t(14;18)(q32;q21), +17[cp8]/46,XY[7]	47~51,XY,+X, add(1)(p36),add(6)(q27), del(6)(q23),del(9)(p22), +12, dup(13)(q21q31), t(14;18)(q32;q21)[cp5]/46,XY[7]	Not performed	Not performed	Not performed	Not performed

One year later, a second recurrence with grade 3B FL was diagnosed. He received six courses of R-CHOP. A third relapse with slow-growing lymph nodes and bone marrow involvement was documented 3 years later and Zevalin was administered. Thirteen years after the initial diagnosis, the patient developed a quickly growing tumor on the left side of the neck. The biopsy showed a diffuse infiltration with large atypical cells with large irregular indented nuclei and eosinophilic cytoplasm with the immunophenotype of histiocytes. The bone marrow showed infiltration by diffuse large B cell lymphoma (DLBCL). The patient was treated with five courses of DHAP, with a rapid response of the cervical tumor. The DLBCL in the bone marrow progressed rapidly and the patient died shortly after the last DHAP course.

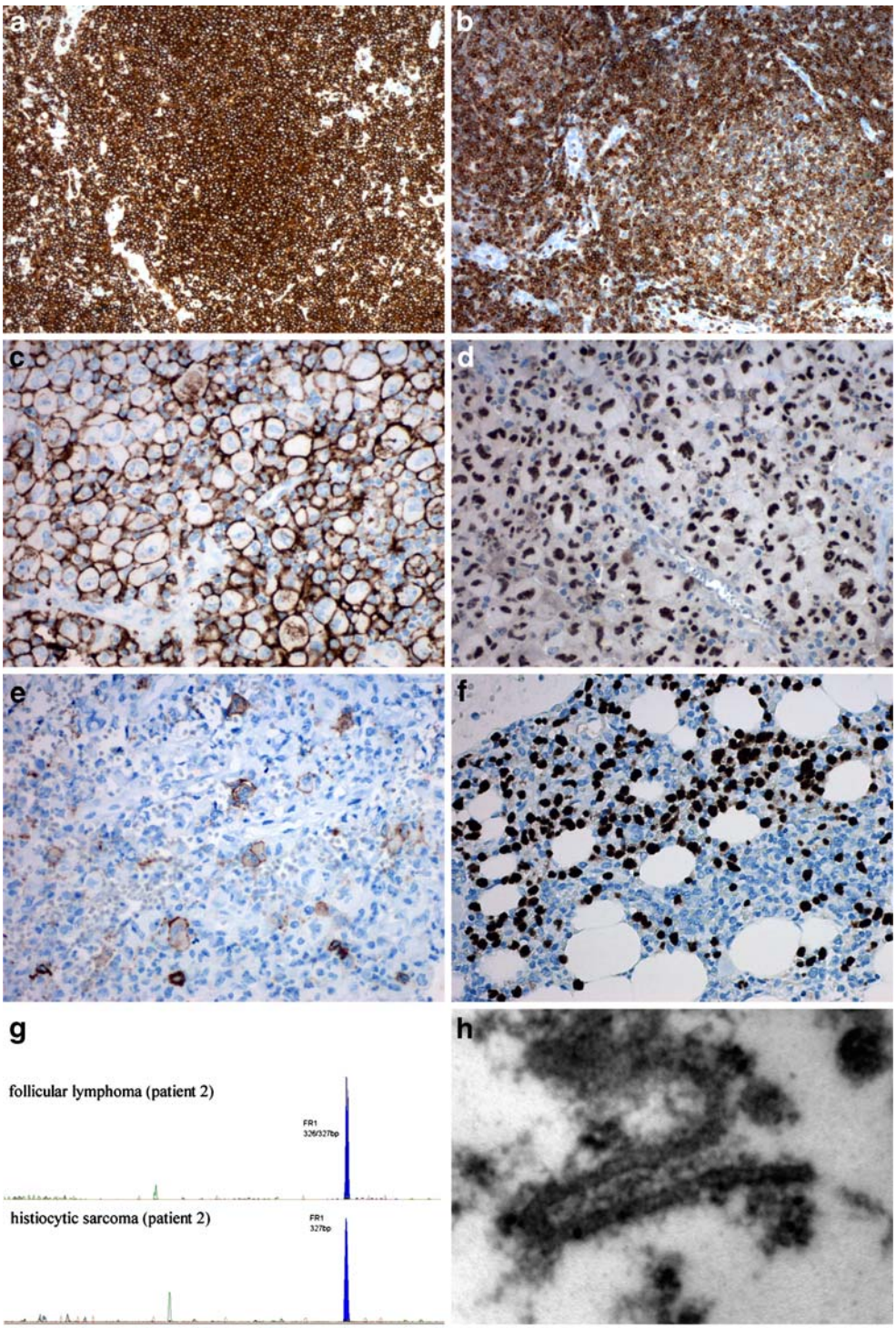

Fig. 2 **a**, **b** Lymph node with follicular lymphoma (CD20 and bcl-2, IHC); **c**, **d**, **e** histiocytic sarcoma (CD14, MAF-B, and CD1a, IHC); **f** bone marrow infiltration with diffuse large B cell lymphoma (PAX-5, IHC); **g** PCR analysis of follicular lymphoma and histiocytic sarcoma; **h** electron microscopy—detail of Birbeck granula (patient 2)

Patient 2 A 62-year-old male patient presented with nodular lesion on the tongue and enlarged cervical lymph nodes and was diagnosed as diffuse large cell B cell lymphoma stage IIA. He received eight courses of R-CHOP-14 with complete remission. One year later, a local relapse with large pleomorphic cells and with the immunophenotype of histiocytic cells was diagnosed. The tumor invaded the base of the tongue, hypopharynx, and cervical lymph nodes. Complete remission after four courses of MIME was achieved and involved field radiotherapy with 40 Gy was given. A second recurrence developed shortly thereafter with similar lesions on the abdomen. The patient received no further treatment and died of tumor progression.

Immunohistochemistry The paraffin blocks were cut at 4–6 μm, dried overnight at 60 °C, and dewaxed in xylene. A standard panel with 16 primary antibodies was used in both cases (Table 1). Visualization was performed using the EnVision® detection system (DAKO Cytomation, Denmark A/S) according to the manufacturer's instructions. Appropriate positive and negative controls were used.

Fluorescent in situ hybridization (FISH) analysis Fluorescent in situ hybridization analysis was performed on formalin-fixed tissue specimens, using a BCL-2 split signal DNA probe (Y5407, DAKO Cytomation, Denmark A/S). The slides were deparaffinized, immersed in pretreatment buffer (K5599, DAKO Cytomation, Denmark A/S) for 10 min at 95°C, followed by pepsin pretreatment, and dehydrated in ethanol. After a denaturation step for 10 min at 80°C, hybridization was performed at 37°C overnight. The next day, the slides were postwashed in saline sodium citrate (SSC) buffer, counterstained with 4′,6-diamidino-2-phenylindole, and evaluated using fluorescent microscope Olympus BX61.

Polymerase chain reaction (PCR) analysis and sequencing DNA was extracted from fresh snap-frozen tissue or paraffin-embedded tissue using QIAmp tissue kit (Qiagen, Hilden, Germany). Analysis of immunoglobulin heavy chain (IgH) gene rearrangement was done using IgH variable gene (VH) family-specific primers (VH1 trough VH6) and VH consensus primers covering the VH framework regions (FR) together with the consensus joining region gene primers as described in the BIOMED-2 protocol [10]. For one sample (case 2, DLBCL), an FR-3-specific primer was used due to the fact that only paraffin-embedded tissue was available. IgH polymerase chain reaction sequences were directly sequenced by using the Big Dye terminator sequencing kit and the Genetic Analyzer 3100 (Applied Biosystems, Weiterstadt, Germany). The sequences obtained were analyzed by comparison with the V base database (www.vbase2.org).

As somatic hypermutation affects only the 5′ region of affected genes, analysis of the 5′ end of the *PAX5* gene was performed using primer pairs covering exon 1 (1A and 1B); exons 2 and 3 were used as previously described [11].

Electron microscopy

Formalin-fixed paraffin-embedded material was used. The paraffin block was cut and the section was immersed in xylene and then rehydrated in alcohol. Then, the material was

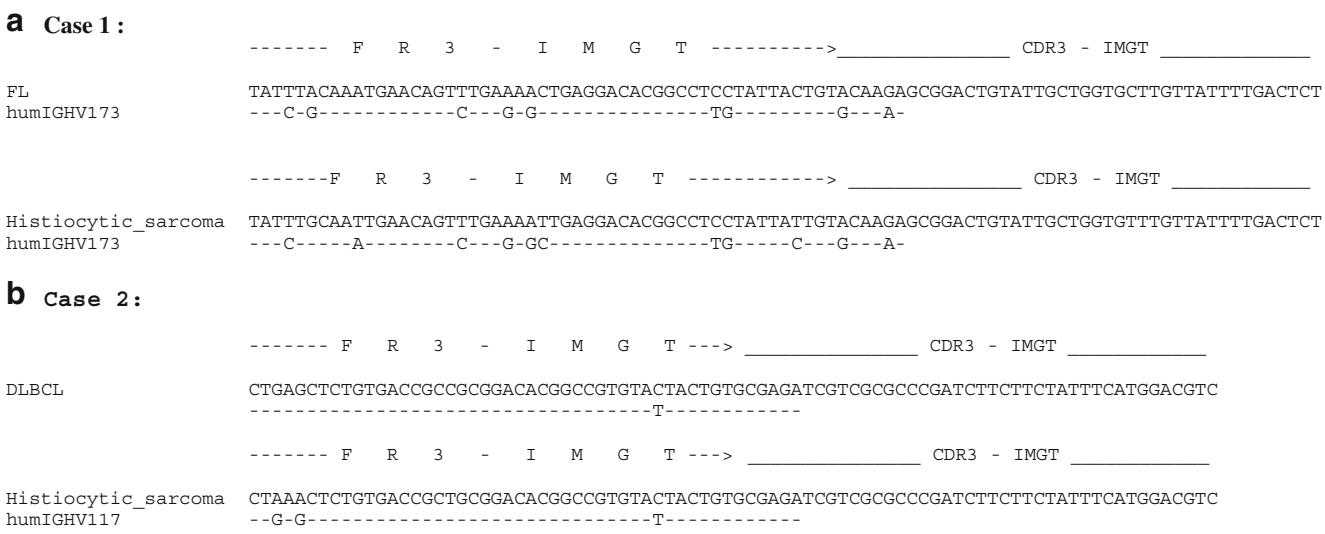

Fig. 3 Sequences of IgH gene rearrangements. The FR3 and CDR3 region sequences are arranged with the identified patient sample at the *top*, compared with the lower V base gene bank sequence with closest homology. *Dashed lines* indicate that the sequence is identical to the gene bank sequence, and a nucleotide indicates difference. **a** Patient 1, follicular lymphoma compared with histiocytic sarcoma. **b** Patient 2, diffuse large B cell lymphoma compared with histiocytic sarcoma

immersed in cacodylate buffer for 24 h and additional postfixation in osmium tetraoxyde for 1 h was performed. After 1 h in uranyl acetate, the tissue was dehydrated, embedded in EPON; ultrathin sections were cut and evaluation was performed using Philips CM10 electron microscope.

Cytogenetics

Fresh tissue samples from patient 1 (two lymph node biopsies and a biopsy from a neck tumor) were manually minced and enzymatically treated until a suitable suspension of cells and cell clumps was obtained. After 5–7 days of culturing, colchicine was added for the last 4 h and the short-term cultures were harvested according to standard protocols. The chromosomes of the dividing cells were then G-banded and a karyotype was established in accordance with the recommendations of the International System for Human Cytogenetic Nomenclature.

Results and discussion

In both patients, B cell lymphoma with a germinal center cell phenotype and t(14; 18) at first presentation was diagnosed. A diagnosis of low-grade follicular lymphoma with a typical morphology and immunophenotype was established on the first biopsy in patient 1 (Fig. 1, Table 2). Patient 2 presented with diffuse large B cell lymphoma with germinal center cell phenotype. In both patients, the initial lymphomas expressed CD20, PAX-5, CD10, and BCL-6 but no histiocytic markers (Fig. 2, Table 2). The recurrence in both patients showed a different histology with large pleomorphic cells with abundant eosinophilic cytoplasm and large irregular nuclei with focal nuclear grooves (Fig. 1, Table 2). The immunophenotype of these recurrences was also different; notably, B cell markers were lost and a strong expression of S100, MAF-B, CD68, CD14, and focal expression of CD1a was documented (Fig. 2, Table 2). Epstein Barr virus-encoded RNA in situ hybridization was negative in both patients. No clinical or morphological evidence of hemophagocytic syndrome was noted in the two patients. Electron microscopy was only successful in patient 2 and showed Birbeck granules (Fig. 2). We found a clonal relationship between the tumors with different phenotypes in both patients as documented by similarly rearranged IgH gene sequences, respectively (Figs. 2 and 3).

Since PAX-5 is crucial for maintaining B cell identity and since the transformed tumors in both patients had lost PAX-5 expression, we sequenced the 5′ part of the *PAX-5* gene to detect probable aberrant somatic hypermutations [9]. Aberrant somatic hypermutation is known to occur in B cell lymphoma and can affect the expression of target genes [8, 12]. However, sequence analysis of the 5′ part of *PAX-5*, including exons 1, 2, and 3 of the *PAX-5* gene, shows an intact structure of the gene without somatic hypermutation. Therefore, aberrant somatic hypermutation of *PAX-5* is an unlikely cause of loss of expression and transdifferentiation at least in our two patients. We have not investigated whether deletion of *PAX-5* gene might explain loss of PAX-5 expression in our two cases. However, this hypothesis seems to be unlikely in view of the fact that the *PAX-5* gene could easily be amplified from DNA extracted from the tumors. Morphological control of the tissues used for DNA extraction assured that the DNA extracts were representative of the tumor and confirmed that a majority of tumor cells were present.

Of interest, cytogenetic data were obtained in patient 1 where the follicular lymphoma as well as the histiocytic sarcoma showed t(14; 18)(18q21). In addition, the recurrent histiocytic sarcoma showed a more complex karyotype with multiple deletions, duplications, and insertions. The latter suggests that chromosomal instability is at least part of the transformation process.

In conclusion, it is of interest that mature neoplastic B cells may display translineage transformation. This seems hitherto restricted to B cell lymphomas of germinal center cell origin with t(14; 18). The mechanisms by which that occur are yet not clear, but aberrant somatic hypermutation of the *PAX-5* gene seems to be an unlikely cause.

Conflict of interest The authors declare that they have no conflict of interest.

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