A novel immunohistochemical classifier to distinguish Hodgkin lymphoma from ALK anaplastic large cell lymphoma

Claudia Döring¹, Martin-Leo Hansmann¹, Claudio Agostinelli², Pier P Piccaluga², Fabio Facchetti³, Stefano Pileri², Ralf Küppers⁴, Sebastian Newrzela¹ and Sylvia Hartmann¹

¹Dr Senckenberg Institute of Pathology, Goethe University Hospital Frankfurt, Frankfurt, Germany;

²Department of Experimental, Diagnostic and Specialty Medicine, Haematopathology Section, S Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy; ³Department of Pathology, University of Brescia, Brescia, Italy and ⁴Institute of Cell Biology (Cancer Research), Faculty of Medicine, University of Duisburg-Essen, Essen, Germany

Classical Hodgkin lymphoma and ALK[−] anaplastic large cell lymphoma share many features like strong CD30 expression and usually loss of B- and T-cell markers. However, their clinical course is dramatically different with curability rates of >90% for classical Hodgkin lymphoma and an unfavorable prognosis for anaplastic large cell lymphoma. Classical Hodgkin lymphoma and ALK[−] anaplastic large cell lymphoma can usually be distinguished by PAX5 expression in the Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma and expression of cytotoxic molecules in tumor cells of anaplastic large cell lymphoma. However, in some cases the differential diagnosis is difficult owing to absence of established markers. To be able to better classify these cases, we reevaluated gene expression data of microdissected tumor cells of both lymphomas for differentially expressed genes. A classifier was established, comprising four genes strongly expressed in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma (*MDC/CCL22*, *CD83*, *STAT3*, and *TUBB2B*). Applying this classifier to a test cohort, Hodgkin lymphoma was successfully distinguished from ALK[−] anaplastic large cell lymphoma with an accuracy of 97% (43/44). *MDC/CCL22*, *CD83*, and *STAT3* have also been found to be expressed in antigen-presenting cells. Therefore, based on our established classifier, Hodgkin and Reed-Sternberg cells differ from tumor cells of anaplastic large cell lymphoma, which can successfully be applied for practical purposes in histopathologic diagnostics.

Modern Pathology (2014) 27, 1345–1354; doi:10.1038/modpathol.2014.44; published online 14 March 2014

Keywords: anaplastic large cell lymphoma; classical Hodgkin lymphoma; classifier; immunohistochemistry

Classical Hodgkin lymphoma and anaplastic large cell lymphoma are both characterized by the presence of CD30-positive tumor cells.^{1,2} Furthermore, anaplastic large cell lymphoma is divided into ALK⁺ cases, presenting with a translocation affecting the ALK locus,^{3–5} and ALK[−] cases, in which translocations involving *DUSP22* have been described in a fraction of cases.⁶ Whereas classical Hodgkin lymphoma often presents in young adults,² anaplastic large cell lymphoma arises at various

ages. ALK⁺ anaplastic large cell lymphoma is more frequently found in children and adolescents, whereas ALK[−] anaplastic large cell lymphoma is often observed in older patients.^{7,8} Classical Hodgkin lymphoma can be cured in about 90% of patients with currently applied treatment approaches.⁹ However, ALK[−] anaplastic large cell lymphoma often shows poor outcome,¹⁰ particularly in the elderly. Therefore, a correct diagnostic classification of both diseases is mandatory for an appropriate therapeutic strategy.

Although classical Hodgkin lymphoma is a B-cell neoplasm, the tumor cells, Hodgkin, and Reed-Sternberg cells—have largely lost their B-cell phenotype.^{11,12} Anaplastic large cell lymphoma is a T-cell neoplasm, but the tumor cells often present with a null phenotype and can be negative for all

Correspondence: Dr S Hartmann, MD, Dr Senckenberg Institute of Pathology, Goethe University Hospital Frankfurt, Theodor-Stern-Kai 7, Frankfurt D- 60590, Germany.
E-mail: s.hartmann@em.uni-frankfurt.de
Received 2 December 2013; revised 25 January 2014; accepted 26 January 2014; published online 14 March 2014

T-cell markers.^{13,14} In ALK⁺ anaplastic large cell lymphoma immunohistochemical detection of ALK expression is a decisive marker for the differential diagnosis, but this marker cannot be applied for ALK⁻ anaplastic large cell lymphoma. In some cases of anaplastic large cell lymphoma cytotoxic molecules like TIA1, perforin, and granzyme B are expressed.¹⁵ However, there are rare Hodgkin lymphoma cases in which expression of these cytotoxic molecules can also be found in Hodgkin and Reed-Sternberg cells.^{15,16} In the past years, the B-cell transcription factor *PAX5* has been discovered to be still expressed at low level in Hodgkin and Reed-Sternberg cells of most classical Hodgkin lymphoma cases.^{11,17,18} Classical Hodgkin lymphoma can therefore in most cases reliably be distinguished from ALK⁻ anaplastic large cell lymphoma. However, some *PAX5*-negative classical Hodgkin lymphoma cases¹⁸ are difficult to be distinguished from ALK⁻ anaplastic large cell lymphoma. Ancillary molecular studies showing rearrangements of B- or T-cell receptors can be helpful in these cases. However, owing to a low tumor cell content, mostly observed in Hodgkin lymphoma and sometimes also found in anaplastic large cell lymphoma, they do not always give indicative results.

Therefore, the aim of the present study was to identify a set of characteristic markers, which can successfully be applied in the differential diagnosis of classical Hodgkin lymphoma and ALK⁻ anaplastic large cell lymphoma.

Materials and methods

Microarray Data Analysis and Selection of Target Genes

Gene expression data from the studies by Brune *et al*¹⁹ and Eckerle *et al*²⁰ were reevaluated. A supervised comparison between ALK⁻ anaplastic large cell lymphoma cases and all Hodgkin lymphoma cases from both studies was performed. After the global filtering, a two-sample *t*-test (assuming equal variance in both groups) was applied to identify genes that were differentially expressed between the two groups. To account for multiple testing, the false discovery rate was used, as described by Benjamini and Hochberg.²¹ For visualization, a heat map of all significantly upregulated or downregulated transcripts was created ($P < 0.05$, false discovery rate < 0.1). Genes to be immunohistochemically validated on the protein level were chosen with regard to the strongest upregulation, the most homogenous expression patterns in the lymphoma samples, and the commercial availability of antibodies.

Tissue Samples and Immunohistochemical Stainings

Thirty classical Hodgkin lymphoma cases (10 nodular sclerosing subtype, 10 mixed cellularity, and 10 Hodgkin lymphoma cases with expression of

granzyme B—9 mixed cellularity and 1 lymphocyte depleted), 20 nodal ALK⁻ anaplastic large cell lymphoma as well as 11 primary cutaneous ALK⁻ anaplastic large cell lymphoma, diagnosed according to the World Health Organization 2008 classification,² were selected from the archives of the Dr Senckenberg Department of Pathology in Frankfurt. We specifically selected granzyme B expressing Hodgkin lymphoma cases, because in classical Hodgkin lymphoma with *PAX5*⁺ Hodgkin and Reed-Sternberg cells sometimes show coexpression of cytotoxic molecules, in particular granzyme B, thus sharing even more features with anaplastic large cell lymphoma. A validation cohort of 30 additional classical Hodgkin lymphoma cases, 13 ALK⁻ and 11 ALK⁺ anaplastic large cell lymphoma cases was selected from the archives of the Department of Experimental, Diagnostic and Specialty Medicine, Haematopathology Section, S. Orsola-Malpighi Hospital, University of Bologna, Italy and stained on tissue microarray format. Stainings were considered positive if at least 50% of the tumor cells were positive. The local ethics committees of the Universities in Frankfurt and Bologna approved the study. Detailed information on the immunohistochemical profiles of the cases are found in Table 1. Whole tissue sections were stained for *CD83*, *MDC/CCL2*, *TUBB2B*, *STAT3*, and *CAPN2* using the Peroxidase-FLEX EnVision Kit (Dako) as described previously.^{22,23} The antibodies used, dilutions, and providers are listed in Table 2. Antigen unmasking was performed for 3 min in a pressure cooker or microwave in Tris-EDTA at pH 8.0 or citrate buffer at pH 6.0. All cases had previously been stained for CD20, CD30, CD3, *PAX5*, TIA1, granzyme B, and LMP1. Hierarchical cluster analysis was conducted on the four immunohistochemical stainings included in the classifier using R and Bioconductor.

Results

Gene Expression Analysis

In a reevaluation of gene expression analyses performed by Brune *et al*¹⁹ and Eckerle *et al*²⁰ a supervised comparison of the gene expression profiles of microdissected tumor cells of classical Hodgkin lymphoma and ALK⁻ anaplastic large cell lymphoma was performed. Eighteen transcripts were found to be overexpressed in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma compared with the tumor cells of ALK⁻ anaplastic large cell lymphoma and 15 transcripts were overexpressed in the tumor cells of ALK⁻ anaplastic large cell lymphoma (fold change < -1.8 or > 1.8 , P -value < 0.05 , false discovery rate < 0.1 , Table 3, Figure 1). Based on significant differential expression, macrophage-derived chemokine/chemokine ligand 22 (*MDC/CCL22*) (39.8-fold upregulated

Table 1 Expression of immunohistochemical markers in different subsets of classical Hodgkin lymphoma and anaplastic large cell lymphoma

Diagnosis	Median age (years)	Male gender (%)	EBV (LMP1)	PAX5	CD20	Granzyme B	TIA1	TUBB2B	STAT3	MDC/CCL22	CD83	CAPN2
cHL, mixed cellularity (WTS)	62.5	50	5/10 (50%)	10/10 (100%)	3/10 ^a (30%)	0/10 (0%)	0/10 (0%)	8/10 (80%)	10/10 (100%)	10/10 (100%)	8/10 (80%)	3/10 (30%)
cHL, nodular sclerosis (WTS)	33	40	0/10 (0%)	10/10 (100%)	3/10 ^a (30%)	0/10 (0%)	0/10 (0%)	8/10 (80%)	7/10 (70%)	10/10 (100%)	10/10 (100%)	2/10 (20%)
cHL, cytotoxic (WTS)	49	70	8/10 (80%)	8/10 (80%)	1/10 ^a (10%)	10/10 (100%)	2/10 (20%)	8/10 (80%)	8/10 (80%)	5/10 (50%)	7/10 (70%)	2/10 (20%)
cHL (TMA)	—	—	—	—	—	—	—	23/30 (77%)	28/30 (88%)	23/30 (77%)	27/30 (90%)	—
HL total	60	85	0/20 (0%)	0/20 (0%)	0/20 (0%)	11/20 (55%)	11/20 (55%)	47/60 (78%)	53/60 (88%)	48/60 (80%)	52/60 (87%)	7/30 (23%)
ALCL, ALK ⁻ (WTS)	67	—	0/11 (0%)	0/11 (0%)	0/11 (0%)	3/7 (43%)	2/4 (50%)	3/20 (16%)	2/20 (11%)	0/20 (0%)	0/20 (0%)	11/20 (55%)
cutaneous ALCL (WTS)	—	—	—	—	—	—	—	1/11 (9%)	1/11 (9%)	0/11 (0%)	0/11 (0%)	3/11 (27%)
ALCL, ALK ⁻ (TMA)	—	—	—	—	—	—	—	1/13 (8%)	2/13 (15%)	0/13 (0%)	4/13 (31%)	—
ALCL, ALK ⁺ (TMA)	—	—	—	—	—	—	—	0/11 (0%)	1/11 (9%)	0/11 (0%)	0/11 (0%)	—
ALCL total	—	—	—	—	—	—	—	5/55 (9%)	6/55 (11%)	0/55 (0%)	4/55 (7%)	14/31 (45%)

Abbreviations: TMA, tissue micro array; WTS, whole tissue section.

^aWeak and heterogeneous CD20 expression.

in Hodgkin and Reed-Sternberg cells), tubulin beta 2B (*TUBB2B*) (9.9-fold upregulated in Hodgkin and Reed-Sternberg cells), *CD83* (8.4-fold upregulated in Hodgkin and Reed-Sternberg cells), signal transducer and activator of transcription 3 (*STAT3*) (4.2-fold upregulated in Hodgkin and Reed-Sternberg cells), and calpain 2 (*CAPN2*) (5.0-fold upregulated in the tumor cells of ALK⁻ anaplastic large cell lymphoma) were selected for immunohistochemical studies (Figure 1).

Immunohistochemical Investigation of Differentially Expressed Genes on Protein Level

In majority of the 30 classical Hodgkin lymphoma cases of the first series, stained on whole tissue sections, Hodgkin and Reed-Sternberg cells were positive for *TUBB2B* (80%) as well as *STAT3*, *CD83*, and *MDC/CCL22* (83% each, Table 1 and Figure 2). *MDC/CCL22* and *CD83* were strongly expressed in the cytoplasm and Golgi field of Hodgkin and Reed-Sternberg cells, whereas *TUBB2B* showed a homogeneous expression in the cytoplasm. An enhanced *STAT3* expression was observed in the nucleus and cytoplasm of Hodgkin and Reed-Sternberg cells. A weak expression of *STAT3* was also observed in histiocytes and the tumor cells of some anaplastic large cell lymphoma cases (Figures 2e and f). Only 10% of the 31 anaplastic large cell lymphoma in the first series presented with a strong nuclear and cytoplasmic *STAT3* expression and cytoplasmic *TUBB2B* expression. Like the Hodgkin and Reed-Sternberg cells, a subgroup of macrophages/dendritic cells stained positive for *CD83* and *MDC/CCL22* in some cases, whereas anaplastic large cell lymphoma tumor cells were consistently negative in this first series. Interestingly, among 10 classical Hodgkin lymphoma cases with expression of granzyme B, Hodgkin and Reed-Sternberg cells were less frequently positive for the Hodgkin classifier markers, when compared with the typical classical Hodgkin lymphoma cases being negative for cytotoxic molecules. In these cases, *MDC/CCL22* was positive in only 5/10 cases (50%), *CD83* was positive in 7/10 cases (70%), *TUBB2B* and *STAT3* were each positive in 8/10 cases (80%).

CAPN2 was expressed in the tumor cells of both classical Hodgkin lymphoma and ALK⁻ anaplastic large cell lymphoma cases (23 and 45%, respectively) and was therefore not further considered.

In the training set, stained on tissue microarray, Hodgkin and Reed-Sternberg cells were also positive in majority of the 30 classical Hodgkin lymphoma cases for *TUBB2B* and *MDC/CCL22* (77% each), *STAT3* (93%), and *CD83* (90%). In contrast, tumor cells of the 13 ALK⁻ anaplastic large cell lymphoma cases were only infrequently positive for *TUBB2B* (8%), *STAT3* (15%), and *CD83* (31%). They were always negative for *MDC/CCL22*.

Table 2 Antibodies and dilutions applied for immunohistochemistry

Antibody	Dilution	Pretreatment	Detection system	Provider
CD83 UCH-B1	1:10	PC pH8 EDTA	2	Novus Biologicals, Cambridge, UK
MDC/CCL22 ab9847	1:50	PC pH8 EDTA	2	Abcam, Cambridge, UK
TUBB2B LS-B4190	1:50	PC pH8 EDTA	2	Lifespan Biosciences, Seattle, USA
STAT3 sc-482	1:100	MW pH6 Citrate	2	Santa Cruz Biotechnology, Santa Cruz, USA
CAPN2 sc-373966	1:200	MW pH6 Citrate	2	Santa Cruz Biotechnology, Santa Cruz, USA
CD30	1:100	PC pH8 EDTA	1	Dako, Glostrup, Denmark
TIA1	1:200	PC pH8 EDTA	1	Immunotech, Monrovia, Canada
Granzyme B	1:100	PC pH8 EDTA	1	Dako, Glostrup, Denmark
PAX5	1:200	PC pH8 EDTA	1	Dako, Glostrup, Denmark
LMP1	1:200	PC pH8 EDTA	2	Dako, Glostrup, Denmark
CD20	1:1000	PC pH8 EDTA	1	Dako, Glostrup, Denmark

Abbreviations: MW, microwave; PC, pressure cooking.

For detection/visualization either ¹K5005 or ²Envision-FLEX, both DAKO, were applied.

Table 3 Transcripts differentially upregulated and downregulated in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma compared with tumor cells of ALK⁻ anaplastic large cell lymphoma^a

Fold change	Gene symbol	Gene name
39.8	CCL22	Chemokine (C-C motif) ligand 22
26.4	MMP12	Matrix metalloproteinase 12 (macrophage elastase)
11.7	CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)
9.9	TUBB2B	Tubulin, beta 2B
9.1	PTGDS	Prostaglandin D2 synthase 21 kDa (brain) /// prostaglandin D2 synthase 21 kDa (brain)
8.4	CD83	CD83 molecule
8.2	—	—
7.6	IL1R2	Interleukin 1 receptor, type II
5.3	MCOLN2	Mucopolipin 2
4.7	SLC2A6	Solute carrier family 2 (facilitated glucose transporter), member 6
4.5	EBI3	Epstein-Barr virus induced gene 3
4.2	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)
3.9	STAT5A	Signal transducer and activator of transcription 5A
3.8	SOCS3	Suppressor of cytokine signaling 3
3.7	APBB1IP	Amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
3.6	RAB13	RAB13, member RAS oncogene family
3.5	BASP1	Brain abundant, membrane attached signal protein 1
3.4	IER3	Immediate early response 3
-1.9	HIST1H4C	Histone 1, H4c
-2.0	—	—
-2.2	—	CDNA FLJ37566 fis, clone BRCOC2002085
-2.2	EEF1D	Eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)
-2.3	RPS27	Ribosomal protein S27 (metalloproteinase 1)
-2.3	RPS10	Ribosomal protein S10
-2.5	RNPS1	RNA binding protein S1, serine-rich domain
-2.7	NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14 kDa
-2.9	SAC3D1	SAC3 domain containing 1
-3.2	ATP6V0E	ATPase, H ⁺ transporting, lysosomal 9 kDa, V0 subunit e
-3.4	LOC653784	Similar to hypothetical protein FLJ14346
-3.5	NDUFA4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4, 9 kDa
-3.5	BTBD6	BTB (POZ) domain containing 6
-3.9	—	Transcribed locus, strongly similar to XP_511906.1 PREDICTED: similar to KIAA0612 protein [Pan troglodytes]
-5.0	CAPN2	Calpain 2, (m/II) large subunit

P-value <0.05, false discovery rate <0.1.

^aModified from Eckerle *et al.*²⁰

Establishment of a Classifier to Efficiently Distinguish Classical Hodgkin Lymphoma and ALK⁻ Anaplastic Large Cell Lymphoma

A scoring system (0–4) was created reflecting the number of positive stainings (*MDC/CCL22*, *CD83*,

STAT3, and *TUBB2B*) per case (0, no positive stainings; 4, all four stainings positive). The first case series, stained on whole tissue sections, was evaluated applying this scoring system. A threshold of ≥ 2 -positive stainings proved to be optimal for the classification as Hodgkin lymphoma, whereas cases

Figure 1 Heat map of the genes differentially expressed in the microdissected tumor cells of classical Hodgkin lymphoma and ALK[−] anaplastic large cell lymphoma from the studies by Brune *et al*¹⁹ and Eckerle *et al*.²⁰ NA, not annotated; red color: high expression; black color: intermediate expression; green color: low expression. Genes selected for immunohistochemical studies are highlighted by arrows.

with a score 0–1 were regarded as anaplastic large cell lymphoma. Applying this scoring system to the second series of cases stained on tissue microarray, all 30 classical Hodgkin lymphoma cases were correctly classified and only one out of 13 ALK[−] anaplastic large cell lymphoma cases was misleadingly classified as Hodgkin lymphoma (42/43, 97%, Table 4). Applying the classifier to all cases, two out of 60 Hodgkin lymphoma cases and two out of 34 nodal ALK[−] anaplastic large cell lymphoma were misclassified, yielding an accuracy of 96% (90/94). The misclassified Hodgkin lymphoma cases were two cases with granzyme B expression. In an unsupervised hierarchical clustering by the classifier, two large branches were observed (Figure 3).

Discussion

Several attempts have been made to establish classifiers differentiating between ALK[−] anaplastic large cell lymphoma and peripheral T-cell lymphoma not otherwise specified,^{24–26} but to our knowledge this has not been achieved for differential diagnosis of classical Hodgkin lymphoma and ALK[−] anaplastic large cell lymphoma. The aim of the present study was to find new immunohistochemical markers which can be applied in the

differential diagnosis of ALK[−] anaplastic large cell lymphoma and classical Hodgkin lymphoma in daily routine practice. Our classifier was established by the reevaluation of gene expression studies of microdissected tumor cells and shows a high specificity and sensitivity.

Owing to overexpression of several genes in the Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma we were able to distinguish between ALK[−] anaplastic large cell lymphoma and classical Hodgkin lymphoma. The one gene overexpressed in ALK[−] anaplastic large cell lymphoma did not prove to be diagnostically powerful. One of the genes upregulated in classical Hodgkin lymphoma was *TUBB2B*, a member of the beta-tubulin family, involved in the formation of microtubuli and therefore has a role in several important cellular processes, including mitosis. *TUBB2B* has so far not been described to be upregulated in Hodgkin and Reed-Sternberg cells, whereas another member of the beta-tubulin family, *TUBB3*, was observed to be expressed in both the tumor cells of classical Hodgkin lymphoma and anaplastic large cell lymphoma.²⁷ Recently, refusion of Hodgkin and Reed-Sternberg cells with a persisting microtubule bond between daughter cells could be demonstrated to be an important feature of giant cell formation.²⁸ However, the reason of a selective tubulin overexpression

in the Hodgkin and Reed-Sternberg cells as demonstrated in this study so far remains obscure.

The remaining significantly overexpressed genes are related to the interactions between Hodgkin and Reed-Sternberg cells and their microenvironment.

CD83 is usually expressed in mature dendritic cells²⁹ and triggers $CD4^+$ T-cell maturation, but is also expressed by activated B cells and involved in the modulation of B-cell receptor signaling.³⁰ *CD83* has previously been described to be upregulated in

Figure 2 Immunohistochemical stainings differentiating classical Hodgkin lymphoma and anaplastic large cell lymphoma. (a and b) *CD83*; (c and d) *MDC/CCL22*; (e and f) *STAT3*; (g and h) *TUBB2B*; (i and j) *CAPN2*. (a, c, e and g) Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma show expression of the respective immunohistochemical markers. (b, d, f and h) Tumor cells of ALK-negative anaplastic large cell lymphoma are negative for the respective markers. (i) Hodgkin and Reed-Sternberg cells are negative for *CAPN2*, whereas a subset of lymphocytes is positive. Inset: classical Hodgkin lymphoma case with *CAPN2*-positive Hodgkin and Reed-Sternberg cells. (j) Tumor cells of ALK⁻ anaplastic large cell lymphoma show expression of *CAPN2*.

Figure 2 Continued.

Table 4 Scores obtained in different subsets of classical Hodgkin lymphoma and anaplastic large cell lymphoma applying the scoring system including the immunohistochemical markers TUBB2B, STAT3, MDC, and CD83

	Score 0	Score 1	Score 2	Score 3	Score 4	Total
cHL, mixed cellularity (WTS)	0	0	0	4	6	10
cHL, nodular sclerosis (WTS)	0	0	1	3	6	10
cHL, cytotoxic (WTS)	0	2	1	4	3	10
cHL (TMA)	0	0	5	9	16	30
cHL total	0	2	7	20	31	60
ALCL, ALK ⁻ (WTS)	16	3	1	0	0	20
Cutaneous ALCL (WTS)	8	3	0	0	0	11
ALCL, ALK ⁻ (TMA)	7	5	1	0	0	13
ALCL, ALK ⁺ (TMA)	10	1	0	0	0	11
ALCL total	41	12	2	0	0	55

Abbreviations: TMA, tissue micro array; WTS, whole tissue section.

Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma.³¹ Transgenic overexpression of *CD83* in B cells resulted in an upregulation of the major histocompatibility complex II (MHC II) and interleukin 10.³⁰ However, MHC II is shown to be down-regulated in a subset of classical Hodgkin lymphoma cases,³² partly owing to translocations involving the

MHC II transactivator (*CIITA*).³³ Decreased MHC class II expression has been linked to reduced tumor cell immunogenicity. A strong expression of *STAT3* was observed in Hodgkin and Reed-Sternberg cells in the present and previous studies.^{34–36} *STAT3* is mainly expressed in macrophages and dendritic cells and can exhibit—depending on the stimulating

Figure 3 Unsupervised hierarchical clustering by the classifier of four immunohistochemical stainings for *MDC/CCL22*, *CD83*, *STAT3*, and *TUBB2B*. Red color: tumor cells positive, green color: tumor cells negative. Color code: yellow: classical Hodgkin lymphoma, green: classical Hodgkin lymphoma with granzyme B expression, red: ALK⁻ anaplastic large cell lymphoma, orange: cutaneous anaplastic large cell lymphoma, blue: ALK⁺ anaplastic large cell lymphoma.

cytokine—both pro- and antiinflammatory properties.³⁷ However, *STAT3* is also found to be expressed in ALK⁺ anaplastic large cell lymphoma.³⁸ In contrast, pediatric cases of ALK⁻ anaplastic large cell lymphoma were negative for phosphorylated *STAT3*.³⁹ Our results fit well with the observations made by Piva *et al*,²⁶ when considering the number of ALK⁻ anaplastic large cell lymphoma strongly expressing phosphorylated *STAT3* in most if not all neoplastic cells. This can also be observed on RNA level in the gene expression arrays (Figure 1). However, it is important to point out, that in the present study only the amount of *STAT3* protein was assessed, whereas phosphorylation and activation status were not evaluated. *MDC/CCL22* is usually also expressed by antigen-presenting cells and is upregulated by TH₂-type cytokines.⁴⁰ It was shown to be expressed in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma⁴¹ and age-related Epstein-Barr virus-associated B-cell lymphoproliferative disorders.⁴² *MDC/CCL22* and *TARC/CCL17* share the receptor CCR4, by which TH₂ and regulatory T cells can be attracted by Hodgkin and Reed-Sternberg cells.^{43,44} However, it could recently be shown that the majority of T cells in classical

Hodgkin lymphoma microenvironment represents TH₁ cells.⁴⁵

In conclusion, three of four genes overexpressed in the Hodgkin and Reed-Sternberg cells in comparison with the tumor cells of ALK⁻ anaplastic large cell lymphoma are also expressed in antigen-presenting cells and are related to the interactions between Hodgkin and Reed-Sternberg cells and surrounding T cells. Moreover, Hodgkin and Reed-Sternberg cells are known to express several additional markers usually expressed by antigen-presenting cells.^{46–48} Although Hodgkin and Reed-Sternberg cells have usually lost their B-cell phenotype, they have maintained the features for an interaction with T cells, which is not observed in the tumor cells of ALK⁻ anaplastic large cell lymphoma. Interestingly, Hodgkin lymphoma cases with granzyme B expression were less frequently positive for *MDC/CCL22* and *CD83* than conventional classical Hodgkin lymphoma cases. These cases may therefore differently interact with their microenvironment and may be closely related to anaplastic large cell lymphoma.

In summary, the genes identified in the present study reflect a different pathogenesis of classical

Hodgkin lymphoma and ALK[−] anaplastic large cell lymphoma. By closely interacting with surrounding T cells, Hodgkin and Reed-Sternberg cells have maintained parts of their B-cell identity and show a fundamentally different interaction with their microenvironment than tumor cells in ALK[−] anaplastic large cell lymphoma. These features can be practically applied in the diagnostic distinction between classical Hodgkin lymphoma and ALK[−] anaplastic large cell lymphoma. However, owing to the limited case number investigated in the present study, our newly established classifier should be validated in a large patient series with clinical data.

Acknowledgments

We would like to thank Sabine Albrecht for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (NE-1438/4-1 as part of the FOR-1961 collaborative research group on mature T-cell lymphomas 'CONTROL-T', and KU1315/7-1) and AIRC 5 × 1000 (Grant 10007).

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

- Stein H, Mason DY, Gerdes J, *et al*. The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. *Blood* 1985;66:848–858.
- Swerdlow SH. International Agency for Research on Cancer, World Health Organization. WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. International Agency for Research on Cancer: Lyon, France; 2008, pp 317–334.
- Mason DY, Bastard C, Rimokh R, *et al*. CD30-positive large cell lymphomas ('Ki-1 lymphoma') are associated with a chromosomal translocation involving 5q35. *Br J Haematol* 1990;74:161–168.
- Lamant L, Meggetto F, al Saati T, *et al*. High incidence of the t(2;5)(p23;q35) translocation in anaplastic large cell lymphoma and its lack of detection in Hodgkin's disease. Comparison of cytogenetic analysis, reverse transcriptase-polymerase chain reaction, and P-80 immunostaining. *Blood* 1996;87:284–291.
- Morris SW, Kirstein MN, Valentine MB, *et al*. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* 1994;263:1281–1284.
- Feldman AL, Dogan A, Smith DI, *et al*. Discovery of recurrent t(6;7)(p25.3;q32.3) translocations in ALK-negative anaplastic large cell lymphomas by massively parallel genomic sequencing. *Blood* 2011;117:915–919.
- Savage KJ, Harris NL, Vose JM, *et al*. ALK[−] anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK⁺ ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. *Blood* 2008;111:5496–5504.
- Pileri SA, Agostinelli C, Bacci F, *et al*. Pathobiology of ALK-negative anaplastic large cell lymphoma. *Pediatr Rep* 2011;3(Suppl 2):e5.
- Eichenauer DA, Engert A. Advances in the treatment of Hodgkin lymphoma. *Int J Hematol* 2012;96:535–543.
- Vose J, Armitage J, Weisenburger D. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. *J Clin Oncol* 2008;26:4124–4130.
- Schwering I, Bräuninger A, Klein U, *et al*. Loss of the B-lineage-specific gene expression program in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. *Blood* 2003;101:1505–1512.
- Küppers R, Engert A, Hansmann ML. Hodgkin lymphoma. *J Clin Invest* 2012;122:3439–3447.
- Bonzheim I, Geissinger E, Roth S, *et al*. Anaplastic large cell lymphomas lack the expression of T-cell receptor molecules or molecules of proximal T-cell receptor signaling. *Blood* 2004;104:3358–3360.
- Geissinger E, Sadler P, Roth S, *et al*. Disturbed expression of the T-cell receptor/CD3 complex and associated signaling molecules in CD30⁺ T-cell lymphoproliferations. *Haematologica* 2010;95:1697–1704.
- Foss HD, Anagnostopoulos I, Araujo I, *et al*. Anaplastic large-cell lymphomas of T-cell and null-cell phenotype express cytotoxic molecules. *Blood* 1996;88:4005–4011.
- Asano N, Kinoshita T, Tamaru J, *et al*. Cytotoxic molecule-positive classical Hodgkin's lymphoma: a clinicopathological comparison with cytotoxic molecule-positive peripheral T-cell lymphoma of not otherwise specified type. *Haematologica* 2011;96:1636–1643.
- Foss HD, Reusch R, Demel G, *et al*. Frequent expression of the B-cell-specific activator protein in Reed-Sternberg cells of classical Hodgkin's disease provides further evidence for its B-cell origin. *Blood* 1999;94:3108–3113.
- Agostinelli C, Sabattini E, Gyorret JO, *et al*. Characterization of a New Monoclonal Antibody Against PAX5/BASP in 1525 Paraffin-embedded Human and Animal Tissue Samples. *Appl Immunohistochem Mol Morphol* 2010;18:561–572.
- Brune V, Tiacchi E, Pfeil I, *et al*. Origin and pathogenesis of nodular lymphocyte-predominant Hodgkin lymphoma as revealed by global gene expression analysis. *J Exp Med* 2008;205:2251–2268.
- Eckerle S, Brune V, Döring C, *et al*. Gene expression profiling of isolated tumour cells from anaplastic large cell lymphomas: insights into its cellular origin, pathogenesis and relation to Hodgkin lymphoma. *Leukemia* 2009;23:2129–2138.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Statist Soc* 1995;57:289–300.
- Hartmann S, Tousseyn T, Döring C, *et al*. Macrophages in T cell/histiocyte rich large B cell lymphoma strongly express metal-binding proteins and show a bi-activated phenotype. *Int J Cancer* 2013;133:2609–2618.
- Hartmann S, Agostinelli C, Klapper W, *et al*. Revising the historical collection of epithelioid cell-rich lymphomas of the Kiel Lymph Node Registry: what is

- Lennert's lymphoma nowadays? *Histopathology* 2011; 59:1173–1182.
- 24 Agnelli L, Mereu E, Pellegrino E, *et al.* Identification of a 3-gene model as a powerful diagnostic tool for the recognition of ALK-negative anaplastic large-cell lymphoma. *Blood* 2012;120:1274–1281.
 - 25 Piccaluga PP, Fuligni F, De Leo A, *et al.* Molecular profiling improves classification and prognostication of nodal peripheral T-cell lymphomas: results of a phase III diagnostic accuracy study. *J Clin Oncol* 2013;31:3019–3025.
 - 26 Piva R, Agnelli L, Pellegrino E, *et al.* Gene expression profiling uncovers molecular classifiers for the recognition of anaplastic large-cell lymphoma within peripheral T-cell neoplasms. *J Clin Oncol* 2010;28:1583–1590.
 - 27 Yoon SO, Kim WY, Go H, *et al.* Class III beta-tubulin shows unique expression patterns in a variety of neoplastic and non-neoplastic lymphoproliferative disorders. *Am J Surg Pathol* 2010;34:645–655.
 - 28 Rengstl B, Newrzela S, Heinrich T, *et al.* Re-fusion of small mononucleated Hodgkin cells leads to giant multinucleated Reed-Sternberg cells in Hodgkin lymphoma. *Proc Natl Acad Sci USA* 2013;110:20729–20734.
 - 29 Zhou LJ, Tedder TF. Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily. *J Immunol* 1995;154:3821–3835.
 - 30 Breloer M, Fleischer B. CD83 regulates lymphocyte maturation, activation and homeostasis. *Trends Immunol.* 2008;29:186–194.
 - 31 Sorg UR, Morse TM, Patton WN, *et al.* Hodgkin's cells express CD83, a dendritic cell lineage associated antigen. *Pathology* 1997;29:294–299.
 - 32 Diepstra A, van Imhoff GW, Karim-Kos HE, *et al.* HLA class II expression by Hodgkin Reed-Sternberg cells is an independent prognostic factor in classical Hodgkin's lymphoma. *J Clin Oncol* 2007;25:3101–3108.
 - 33 Steidl C, Shah SP, Woolcock BW, *et al.* MHC class II transactivator CIITA is a recurrent gene fusion partner in lymphoid cancers. *Nature* 2011;471:377–381.
 - 34 Kube D, Holtick U, Vockerodt M, *et al.* STAT3 is constitutively activated in Hodgkin cell lines. *Blood* 2001;98:762–770.
 - 35 Holtick U, Vockerodt M, Pinkert D, *et al.* STAT3 is essential for Hodgkin lymphoma cell proliferation and is a target of tyrosine kinase AG17 which confers sensitization for apoptosis. *Leukemia* 2005;19:936–944.
 - 36 Lamprecht B, Kreher S, Anagnostopoulos I, *et al.* Aberrant expression of the Th2 cytokine IL-21 in Hodgkin lymphoma cells regulates STAT3 signaling and attracts Treg cells via regulation of MIP-3alpha. *Blood* 2008;112:3339–3347.
 - 37 Braun DA, Fribourg M, Sealfon SC. Cytokine response is determined by duration of receptor and signal transducers and activators of transcription 3 (STAT3) activation. *J Biol Chem* 2013;288:2986–2993.
 - 38 Anastasov N, Bonzheim I, Rudelius M, *et al.* C/EBPbeta expression in ALK-positive anaplastic large cell lymphomas is required for cell proliferation and is induced by the STAT3 signaling pathway. *Haematologica* 2010;95:760–767.
 - 39 Nasr MR, Laver JH, Chang M, *et al.* Expression of anaplastic lymphoma kinase, tyrosine-phosphorylated STAT3, and associated factors in pediatric anaplastic large cell lymphoma: a report from the children's oncology group. *Am J Clin Pathol* 2007;127:770–778.
 - 40 Yamashita U, Kuroda E. Regulation of macrophage-derived chemokine (MDC, CCL22) production. *Crit Rev Immunol* 2002;22:105–114.
 - 41 Hanamoto H, Nakayama T, Miyazato H, *et al.* Expression of CCL28 by Reed-Sternberg cells defines a major subtype of classical Hodgkin's disease with frequent infiltration of eosinophils and/or plasma cells. *Am J Pathol* 2004;164:997–1006.
 - 42 Takegawa S, Jin Z, Nakayama T, *et al.* Expression of CCL17 and CCL22 by latent membrane protein 1-positive tumor cells in age-related Epstein-Barr virus-associated B-cell lymphoproliferative disorder. *Cancer Sci* 2008;99:296–302.
 - 43 Marshall NA, Christie LE, Munro LR, *et al.* Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood* 2004;103:1755–1762.
 - 44 Ishida T, Ishii T, Inagaki A, *et al.* Specific recruitment of CC chemokine receptor 4-positive regulatory T cells in Hodgkin lymphoma fosters immune privilege. *Cancer Res* 2006;66:5716–5722.
 - 45 Greaves P, Clear A, Owen A, *et al.* Defining characteristics of classical Hodgkin lymphoma microenvironment T-helper cells. *Blood* 2013;122:2856–2863.
 - 46 Lamprecht B, Walter K, Kreher S, *et al.* Derepression of an endogenous long terminal repeat activates the CSF1R proto-oncogene in human lymphoma. *Nat Med* 2010;16:571–579; 571p following 579.
 - 47 Steidl C, Diepstra A, Lee T, *et al.* Gene expression profiling of microdissected Hodgkin Reed Sternberg cells correlates with treatment outcome in classical Hodgkin lymphoma. *Blood* 2012;120:3530–3540.
 - 48 Hartmann S, Tousseyn T, Döring C, *et al.* Macrophages in T cell/histiocyte rich large B cell lymphoma strongly express metal-binding proteins and show a bi-activated phenotype. *Int J Cancer* 2013;133:2609–2618.