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Original Paper

Relationship between classic Hodgkin lymphoma and overlapping large cell lymphoma investigated by comparative expressed sequence hybridization expression profiling

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Abstract

There is a diagnostic grey zone between classic Hodgkin lymphoma (cHL) and some non-Hodgkin lymphoma (NHL), including primary mediastinal B cell lymphoma, diffuse large B cell lymphoma, and anaplastic large cell lymphoma. They all have some morphological and/or phenotypic features in common. To investigate this, we undertook an expression profiling study of these lymphomas using comparative expressed sequence hybridization. This technique detects chromosomal regions that are differentially expressed between a test and a reference tissue in a manner similar to comparative genomic hybridization, and is particularly suitable when the number of informative biopsies is limited. Using this approach, we identified a unique expression profile for all lymphoma types investigated. Unsupervised hierarchical cluster analysis of the acquired data showed that cHL separates from all investigated NHLs, including ALCL-like HL. Moreover, anaplastic lymphoma kinase (ALK)-negative ALCL clustered in a separate branch together with ALCL-like HL. Thus, analysing the neoplastic cells concurrently with their microenvironment, ALK-negative ALCL and ALCL-like HL seem to be related to each other, while cHL constitutes a separate lymphoma entity.

Keywords: Hodgkin lymphoma; diagnostic grey zone; expression profiling; CESH

Introduction

Since its description by Thomas Hodgkin in the second half of the 19th century, Hodgkin lymphoma (HL) has been considered as a disease separate from non-Hodgkin lymphomas (NHLs) [1]. The most common subtype of HL, classic Hodgkin lymphoma (cHL), is composed of a minority of large neoplastic cells, called Hodgkin and Reed–Sternberg (HRS) cells, in a significant background of inflammatory cells. HRS cells are characterized by CD30, CD15, and B cell-specific activation protein (BSAP; PAX5) expression [2,3], and loss of their B cell phenotype [4].

However, despite well-defined morphological, immunophenotypic, and molecular diagnostic criteria, there is a diagnostic grey zone between cHL and some NHL entities [5]. The latter comprise primary mediastinal B cell lymphoma (PMBCL), diffuse large B cell lymphoma (DLBCL), and anaplastic large cell lymphoma (ALCL). Neoplastic cells from PMBCL frequently express CD30 [6], while mediastinal involvement is regularly seen in cHL, most frequently in the nodular sclerosing subtype (NS-cHL) (80% of cases) [1]. On the other hand, the neoplastic cells in some cases of DLBCL and PMBCL in particular may resemble HRS cells morphologically. Moreover, rare DLBCL cases show upregulation of the anaplastic lymphoma kinase (ALK) protein, although this feature was thought to be specific for ALCL [1,7,8].

Indeed, most ALCL cases are ALK-positive, but ALK-negative cases do exist (about one quarter of ALCL cases) [1]. Typically, ALCL is of T/NK cell or null cell phenotype and consists of activated lymphocytes. Initially, four different morphological ALCL variants were described comprising the common [9] (70%), the lymphohistiocytic [10] (10%), the small cell [11] (5–10%) and the Hodgkin-like [12] (only occasional cases) variants. The last of these represents the borderline between ALCL and cHL, two lymphoma entities that show a morphological and immunophenotypic overlap [13,14]. In the Revised European–American Lymphoma (REAL) classification [15], Hodgkin-like ALCL was included as a provisional entity. However, in the more recent World Health Organization (WHO) classification [1], the latter was no longer considered as an entity, since there

is no real biological overlap. Moreover, the frequent expression of PAX5 and the absence of the ALK protein expression in Hodgkin-like ALCL favour the concept that these cases are actually tumour cell-rich true NS-cHL cases and should be named ALCL-like HL [16]. Still, occasional ALCL cases may be associated with sclerosis and more commonly NS-cHL may contain confluent sheets of HRS cells and resemble ALCL [1,15].

The differential diagnosis between cHL and all of the above-mentioned NHLs is clinically important since they currently receive different therapy regimens. DLBCL, PMBCL, and ALCL are treated by a third-generation chemotherapy regimen, as used for high-grade NHL, while for cHL a scheme specific for this lymphoma type is administered [17–20]. Of interest, for ALCL-like HL, similar complete remission and relapse-free survival occur with both kinds of treatment [21].

In an attempt to determine the relationship between cHL and some NHLs showing an overlapping morphology, we undertook an expression profiling study using comparative expressed sequence hybridization (CESH). This recently developed technique identifies chromosomal regions of differential expression in a way similar to that of comparative genomic hybridization (CGH) [22]. Previously, we successfully adapted this technique for use in the molecular investigation of lymphomas and of breast cancer [23,24].

Materials and methods

Study group

The patients included in this study were diagnosed at the University Hospitals of Leuven between 1985 and 2001. We selected cases on the basis of the availability of fresh-frozen diagnostic biopsy material and included in our study a total of 25 representative cases divided between the investigated lymphoma types as follows: NS-cHL of grade 2 according to the British National Lymphomas Investigation (BNLI) grading system [25] (cases 1–4), ALCL-like HL (cases 5–8), systemic ALK-negative ALCL (cases 9–11), systemic ALK-positive ALCL (cases 12–15), DLBCL of germinal centre-like phenotype (GC-DLBCL) with t(14;18)(q32;q21) (cases 16–19), DLBCL of activated B cell-like phenotype (ABC-DLBCL) (cases 20–23), and PMBCL (cases 24, 25).

All cases were morphologically reviewed and the diagnosis of NS-cHL, ALK-positive ALCL, DLBCL, and PMBCL was made according to criteria of the WHO classification [1]. Although tumour cells are generally scarce in cHL, it should be emphasized that NS-cHL grade 2 subtype cases contain rather high numbers of HRS cells [1]. We selected particularly those NS-cHL cases with at least 25% of HRS cells expressing CD30, CD15, and PAX5.

ALK-negative ALCL cases were composed of large sheets of medium to large tumour cells growing in a diffuse or interfollicular pattern. HRS-like cells and large, highly pleomorphic cells were present. The atypical cells expressed CD30 but did not express CD15 or PAX5. ALCL-like HL on the contrary, was characterized by a more pronounced fibrotic and inflammatory background. These tumours were composed of large syncytial sheets of HRS-like cells, expressing CD30, CD15, and PAX5.

Table 1 summarizes the clinical characteristics of all cases included in this study. The majority of the patients were treated using anthracycline-containing chemotherapy, except for cases 5, 9, and 10 who received a chemotherapy regimen without anthracycline and case 21 who received no treatment at all.

Additionally, four patients with reactive lymph nodes were enrolled into our study to serve as reference material. These lymph nodes showed no obvious morphological anomalies on haematoxylin and eosin (H&E) stained sections.

Our study was approved by the institutional ethics commission of the K.U. Leuven.

RNA preparation and reverse transcription

Total RNA was isolated from eight 20 μm sections of each frozen tissue sample using the Rneasy Mini Kit (Qiagen, Venlo, The Netherlands) following the manufacturer's recommendations. The quality and concentration of the RNA were measured spectrophotometrically. Total RNA (1 μg) was reverse transcribed into cDNA using random hexamers and superscript II (Life Technologies, Merelbeke, Belgium).

Complementary DNA pooling

For each of the investigated lymphoma types, we pooled equal amounts of cDNA obtained from the individual cases of the same lymphoma type. Care was taken that the individual cases within each pool displayed similar clinical characteristics (Table 1). A pool of reactive lymph nodes was prepared in a similar way.

Complementary DNA labelling

The cDNA from individual cases and pooled cDNA were amplified and differentially labelled with SpectrumGreen-dUTP or SpectrumRed-dUTP (Abbott, Ottignies, Belgium) during two rounds of degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) using the degenerate primer UN-1 [26]. Labelled probes were purified using Qiaquick PCR purification columns (Qiagen). Label incorporation was checked by the Nanodrop spectrophotometer (Isogen Lifescience, Ijsselstein, The Netherlands) and varied between 25 and 50 pmol labelled dUTP per microgram cDNA.

Comparative expressed sequence hybridization (CESH)

For our study, the number of representative cases documented with frozen tissue, as recommended for gene

Table 1. Clinical characteristics, and CESH study design, of all lymphoma cases included in this study

Case	Diagnosis*	Patient age/sex	Stage†	IPI‡	Current status§ (years follow up)	CESH study design¶
1	NS-cHL	9 M	IIA	L	CR (7)	E2/E3
2	NS-cHL	17 F	IIA	L	CR (10)	
3	NS-cHL	15 F	IIB	L	CR (5)	
4	NS-cHL	10 M	IIIA	LI	CR (5)	
5	ALCL-like HL	12 M	IVB	LI	Died, NR (<1)	E1/E2/E3
6	ALCL-like HL	76 F	NA	NA	NA	
7	ALCL-like HL	15 F	IIIA	L(I)	CR (9)	
8	ALCL-like HL	59 M	IVA	HI	Died, NR (1)	
9	ALK-negative ALCL	59 M	IVA	LI	Died (<1)	E1/E2/E3
10	ALK-negative ALCL	82 M	IEB	L	NR (<1)	
11	ALK-negative ALCL	71 M	IIIA	LI	CR (1)	
12	ALK-positive ALCL	9 M	IVA	L	CR (4)	E1/E2
13	ALK-positive ALCL	9 F	IIA	L	CR (4)	
14	ALK-positive ALCL	33 F	IIIB	L	CR (<1)	
15	ALK-positive ALCL	25 M	IA	L	CR (9)	
16	GC-DLBCL	59 F	IV	LI	Died (9)	E1/E2
17	GC-DLBCL	59 F	IV	HI	Died (2)	
18	GC-DLBCL	47 M	IV	HI	Died (<1)	
19	GC-DLBCL	51 F	I	L	CR (11)	
20	ABC-DLBCL	57 F	IV	LI	Died (2)	E1/E2
21	ABC-DLBCL	74 F	IE	L	Died (7)	
22	ABC-DLBCL	79 F	NA	NA	NA	
23	ABC-DLBCL	34 F	NA	NA	NA	
24	PMBCL	30 F	IIE	L	CR (1)	E1/E2
25	PMBCL	33 F	IV	LI	Died (<1)	

NA, no information available.

* NS-cHL, classic Hodgkin lymphoma nodular sclerosis type; ALCL, anaplastic large cell lymphoma; ALCL-like HL, ALCL-like Hodgkin lymphoma; ALK-negative ALCL, anaplastic lymphoma kinase-negative ALCL; ALK-positive ALCL, anaplastic lymphoma kinase-positive ALCL; GC-DLBCL, germinal centre-like diffuse large B cell lymphoma; ABC-DLBCL, activated B cell-like DLBCL; PMBCL, primary mediastinal B cell lymphoma.

† According to the Ann Arbor classification.

‡ Prognostic score, according to the International Prognostic Index. Although initially developed and validated for large cell non-Hodgkin lymphomas, we included in the table also the IPI score for all Hodgkin lymphoma cases as a means of clinical comparison. L = low; LI = low–intermediate; HI = high–intermediate.

§ CR, complete remission; NR, no remission.

¶ In a first and a second series of CESH profiling experiments, pooled cDNA from cases of the same lymphoma type was paired with pooled cDNA from four NS-cHL (E1) and pooled cDNA from four reactive lymph nodes (E2) respectively, while in a third series of experiments cDNA from individual NS-cHL, ALCL-like HL and ALK-negative ALCL cases was paired with pooled cDNA from four reactive lymph nodes (E3).

expression profiling, is limited. This is especially true for the ALCL-like HL, which is a rather rare lymphoma type, and for the NS-cHL selected upon a relatively high tumour burden. Since CESH does not detect differentially expressed genes, but rather chromosomal regions, this technique generates a limited set of data points compared with the thousands of genes analysed by microarray expression profiling. From a statistical point of view, CESH thus represents a more acceptable way of expression profiling, generating datasets of statistical significance from the limited number of cases investigated in our study.

CESH was performed according to Lu *et al* [22]. Fluorescently labelled test and reference cDNA were prepared for competitive hybridization onto normal metaphase chromosomes using the CGH hybridization kit (Abbott). Hybridization was carried out in a humid chamber during 72 hours at 37 °C. Ten good quality metaphases, counterstained with 4,6-diamidino-2-phenylindole were analysed in each experiment. Image acquisition and analysis were performed using a Zeiss Axioplan 2 fluorescence microscope (Zeiss, Zaventem, Belgium) equipped with a cooled charge-coupled device camera COHU 4910 (Diagnostic Instruments, Detroit, MI, USA) and controlled by Cytovision software v2.81 (Applied Imaging International, Newcastle upon Tyne, UK). Dynamic standard reference intervals (SRIs) were used as thresholds for determination of relative over- and underexpression [27]. These SRIs were created in our system as previously described [24] and are based upon systematic ratio variations seen in normal samples. We considered as biologically relevant only those regions detected at the 95% confidence interval (CI). This value, applied to record chromosomal regions with a differential expression, is a recognized statistical indication of how accurately the obtained profile reflects the real aberrations in the sample.

CESH data analysis

The acquired CESH data were further interpreted by unsupervised hierarchical cluster analysis in an attempt to reveal correlated gene expression patterns that illustrate the relationship between the investigated cases. For this purpose, all overexpressed regions detected at the 95% CI by CESH analysis were designated 1, while all underexpressed regions were designated −1. These transformed data were imported into the Multiexperiment Viewer, one of the four applications of the TM4 suite developed by The Institute for Genomic Research that is freely available at http://www.tigr.org/software/(accessed 5 July 2006) [28]. Average linkage clustering was performed applying the Euclidean distance metric.

Study design

Table 1 gives an overview of the study design.

To study the relationship between NS-cHL and ALCL-like HL, ALK-negative ALCL, ALK-positive ALCL, PMBCL, GC-DLBCL, and ABC-DLBCL at the gene expression level, relative CESH profiles were initially determined using a pool of four NS-cHL as reference material and a pool of cases of each of the above mentioned NHL (see 'Study group') as test material.

In the next step, relative CESH profiles of pools of all of the above lymphomas were determined pairing them with a pool of four reactive lymph node biopsies as reference material. Using this approach, we could validate our findings generated from the first set of experiments, and we further investigated the CESH expression profile of NS-cHL in relation to the profiles of the investigated NHLs.

The pooling of several cases of each of the investigated lymphoma types was done to reduce inter-individual variability in the gene expression profiles.

To check whether the acquired CESH profiles of the pooled samples were representative for the respective investigated entities, all individual NS-cHL, ALCL-like HL, and ALK-negative ALCL cases were CESH profiled versus the above pool of four reactive lymph nodes.

Results

In general, the detection of differentially expressed regions by CESH throughout this study was reproducible, as confirmed in duplicate experiments (data not shown). Moreover, numerous chromosomal regions showing differential expression (ranging between 8 and 109 per expression profile) have been identified throughout this study.

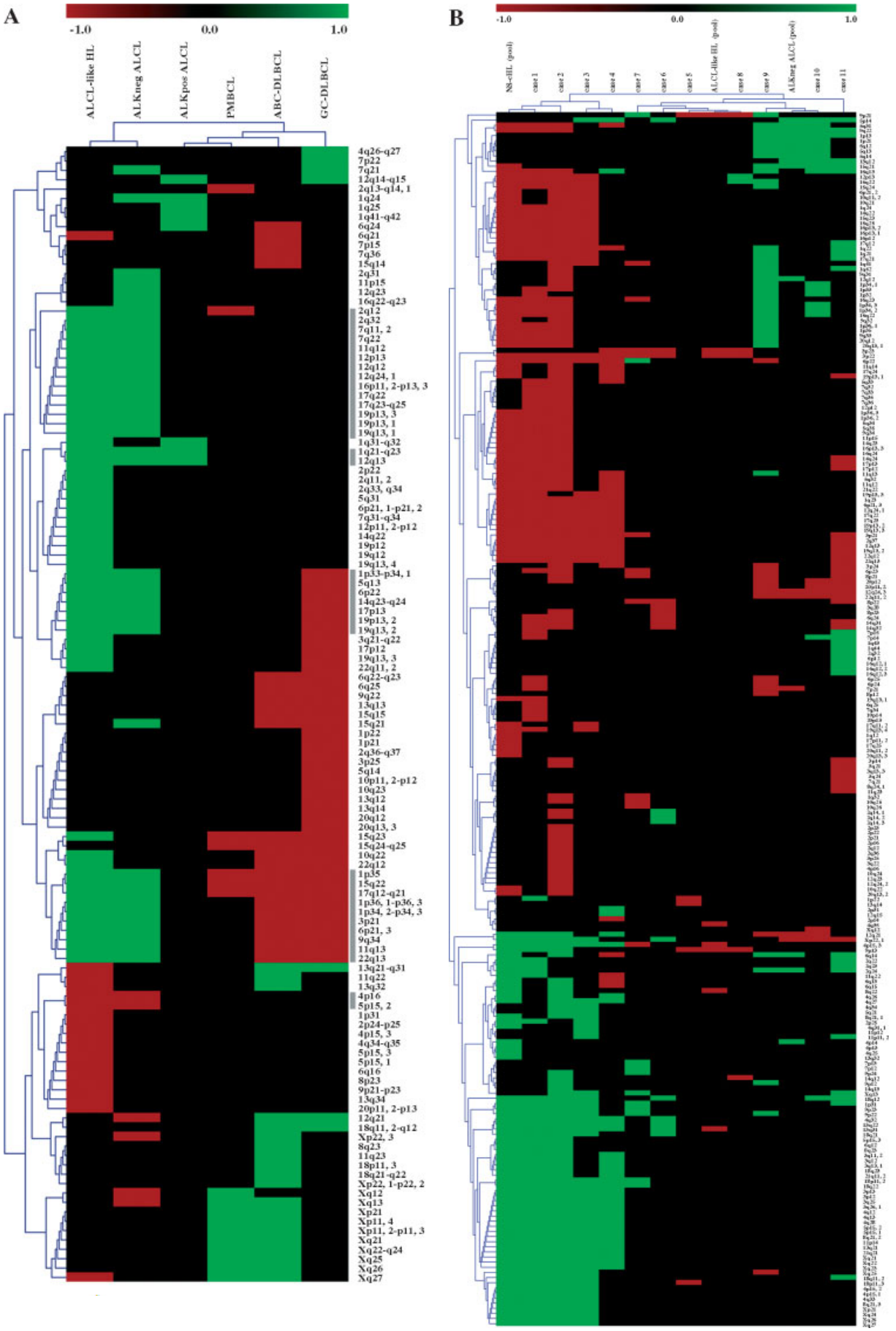
In the first step of our study, a CESH analysis which paired pools of respectively ALCL-like HL, ALK-negative ALCL, ALK-positive ALCL, PMBCL, ABC-DLBCL, and GC-DLBCL with a pool of NS-cHL was performed. The lymphoma types showing the least differentially expressed regions in comparison with NS-cHL included ALK-positive ALCL (eight regions) and PMBCL (17 regions). The remaining lymphomas, ABC-DLBCL, ALK-negative ALCL, GC-DLBCL, and ALCL-like HL showed respectively 45, 46, 49, and 70 chromosomal regions of differential expression in their relative expression profiles.

Figure 1A shows the dendrogram resulting from an unsupervised hierarchical cluster analysis of the CESH profiles of this first series of experiments. The main branches of this dendrogram comprised ALK-positive ALCL, PMBCL, ABC-DLBCL, and GC-DLBCL on one branch and ALCL-like HL and ALK-negative ALCL on the other branch. Figure 1A shows the common signature registered for the last two, comprising 33 chromosomal regions showing relative overexpression and 2 showing relative underexpression.

In the next step of our study, we paired a pool of each of the investigated lymphoma types, including NS-cHL, with a pool of reactive lymph nodes. After unsupervised hierarchical cluster analysis (see Supplementary Figure at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2043.html), the resulting CESH profiles showed a dendrogram similar to that obtained in the first series of experiments. Strikingly, NS-cHL immediately detached itself from all investigated NHLs. Those lymphoma types presenting the least differentially expressed chromosomal regions comprise ALCL-like HL (eight regions), ALK-negative ALCL (19 regions), and to a lesser extent ALK-positive ALCL (63 regions). The remaining investigated lymphoma types all showed a markedly higher number (around 100 regions) of differentially expressed chromosomal regions in comparison with reactive lymph node tissue.

To verify the validity of the acquired results the latter experiments were repeated, this time by pairing the individual cases composing the investigated pools

Figure 1. Unsupervised hierarchical cluster analysis of CESH data. Only those CESH regions registered at the 95% confidence interval (CI) were included for further unsupervised hierarchical cluster analysis. Each column represents one hybridization experiment and each row represents an informative differentially expressed chromosomal region. Relative over- and underexpression are depicted in green and red, respectively, while black squares represent chromosomal regions showing no differential expression. (A) Relative CESH expression profiles were established by pairing pools of ALCL-like HL, ALK-negative ALCL (ALKneg ALCL), ALK-positive ALCL (ALKpos ALCL), PMBCL, ABC-DLBCL, and GC-DLBCL with a pool of NS-cHL. Unsupervised hierarchical cluster analysis results in one branch comprising ALCL-like HL and ALK-negative ALCL while the other branch contains the remaining investigated lymphoma types. The chromosomal regions composing the common signature of ALCL-like HL and ALK-negative ALCL are indicated by grey bars on the right of the dendrogram. (B) Relative CESH expression profiles were established by pairing pools as well as individual cases of NS-cHL (cases 1–4), ALCL-like HL (cases 5–8), and ALK-negative ALCL (cases 9–11) with a pool of reactive lymph nodes. After unsupervised hierarchical cluster analysis it is evident that the data acquired for the pools are representative of the respective investigated lymphoma types



with a pool of reactive lymph nodes. Since the differential diagnosis between NS-cHL, ALCL-like HL, and ALK-negative ALCL is the most challenging, we chose to investigate in particular those individual cases. Figure 1B shows the dendrogram resulting from an unsupervised hierarchical cluster analysis, performed on the CESH data pairing the individual cases as well as the pooled samples with a pool of reactive lymph nodes. Of note, each pooled sample clustered together with its respective individual cases. Moreover, NS-cHL immediately separated itself in one main branch from both ALCL-like HL and ALK-negative ALCL, both clustering together in the other main branch of the dendrogram.

Discussion

We present here the results of a CESH analysis of NS-cHL, ALCL-like HL, ALK-positive ALCL, ALK-negative ALCL, PMBCL, GC-DLBCL, and ABC-DLBCL, undertaken to investigate the relationship between these different lymphoma entities at the expression level.

Although several regions of differential expression were registered, it has to be noted that certain regions coding for genes with known overexpression, such as 2p23/*ALK* in ALK-positive ALCL and 18q21/*BCL2* in the GC-DLBCL with t(14;18)(q32;q21), did not show differential expression. Such single gene expression alterations may easily be registered when analysing homogeneous cell line material [24] but are hard to detect by CESH when analysing tissue samples, as is the case in this study. After all, the gene expression profiles of the investigated tissue samples acquired by CESH represent the mean of the expression profiles of all cell types present in the sample and not only that of the neoplastic cells. The expression profile of the reactive tumour microenvironment may thus mask part of the particular profile of a particular cell type.

Nevertheless, the validity of our CESH profiling approach is illustrated by matches of differentially expressed chromosomal regions with the localization of several genes documented to be differentially expressed in the same lymphoma subtypes, as listed in Table 2 [29–36]. In addition, several differentially expressed regions registered for GC-DLBCL, ABC-DLBCL, and PMBCL correlate with chromosomal aberrations previously identified by Bea and colleagues [37] as discriminating between these lymphoma subtypes using CGH. These include loss of 6q21–q22 and gain of 18q21–q22 (*MALT1* and *BCL2*) in ABC-DLBCL, gain of 12q14 (*CDK4* and *SAS*) in GC-DLBCL, and gain of Xp as a general phenomenon for all three lymphoma subtypes.

Interestingly, several regions almost exclusively overexpressed by ALCL-like HL encode genes belonging to the 'Immune Response 1 signature', identified previously in follicular lymphoma [38]. That signature represents a prognostically favourable gene set consisting mainly of genes highly encoded by T lymphocytes and macrophages (eg *TNFRSF1B, TNFRSF25, STAT4, C1RL, BIN2*). The prognostic impact for ALCL-like HL of this finding is, however, unclear.

One way of evaluating the relatedness of the investigated lymphoma types to cHL would be to consider the number of differentially expressed regions. Using this criterion, ABC-DLBCL (45 regions) and GC-DLBCL (49 regions) showed a much higher number of CESH regions than PMBCL, which showed only 17 CESH regions. These findings remain in agreement with the recently published microarray data, which indicated that PMBCL constitutes a separate entity documented by an expression profile that differs from that of other DLBCLs and shares some features with that of cHL [32]. Using the same criterion, the highest similarity, despite their different cell of origin, was noted between the ALK-positive ALCL and NS-cHL (eight CESH regions). One explanation for these findings might be the loss of the B cell phenotype in cHL and lack of expression of pan-T cell markers in ALCL, as previously reported [4,39].

An alternative method to evaluate the relation between the investigated lymphoma types is unsupervised hierarchical cluster analysis of the obtained CESH data. Notably, the investigated lymphomas did

Table 2. Comparison of CESH profiling data with previously published molecular profiling data

Lymphoma entity*	Gene	Chromosomal region
ABC-DLBCL	*PRKCD* ↓	3p21 ↓
	IER3 ↓	6p21.3 ↓
	TNFAIP3 ↓	6q23 ↓
	SOD2 ↓	6q25 ↓
	CCL2 ↓, *CCL3* ↓, *CCL4* ↓	17q12 ↓
	APR ↑, *BCL2* ↑, *TCF4* ↑	18q21 ↑
	PIM2 ↑	Xp11.2 ↑
	NIK ↑	Xq22 ↑
GC-DLBCL	*SP100* ↓, *LYSP100* ↓, *SP140* ↓, *PASK* ↓	2q37 ↓
	SH3BP5 ↓, *RAFTLIN* ↓	3p25 ↓
	PRKCD ↓	3p21 ↓
	NET ↓	3q22 ↓
	IER3 ↓	6p21.3 ↓
	TNFAIP3 ↓	6q23 ↓
	SOD2 ↓	6q25 ↓
	VIM ↓	10p12 ↓
	FUT8 ↓	14q23 ↓
	BATF ↓	14q24 ↓
	P2RX5 ↓	17p13 ↓
	CCL2 ↓, *CCL3* ↓, *CCL4* ↓	17q12 ↓
	CD23A ↓	19p13.2 ↓
	GADD34 ↓, *SPIB* ↓	19q13.3 ↓
PMBCL	*TIMP1* ↑	Xp11.3 ↑
ALK-positive ALCL	*MCL1* ↑	1q21 ↑
	IRTA2 ↑	1q23 ↑

* ABC-DLBCL, activated B cell-like diffuse large B cell lymphoma; GC-DLBCL, germinal centre-like DLBCL; PMBCL, primary mediastinal B cell lymphoma; ALK-positive ALCL, anaplastic lymphoma kinase-positive anaplastic large cell lymphoma.

not cluster according to their derivation from either B or T cells. For instance, ALK-negative ALCL and ALK-positive ALCL, the only T cell derived NHLs included in our study and thought to represent variants of the same lymphoma type, clustered in different branches of the dendrogram. This may indicate that their expression profiles as established by CESH are not primarily determined by their cell of origin. We assume that the inflammatory background, which makes up a significant portion of the NS-cHL, ALCL-like HL, and ALK-negative ALCL biopsies, is responsible for a considerable part of the CESH profiles in these lymphoma types. This would be analogous to the conclusions drawn by Dave *et al* [38] and Monti *et al* [40] from their microarray analyses of respectively follicular lymphoma and DLBCL, about the importance of the stromal component in their expression profiles. Following this pattern of thinking, the ALCL-like HL and the ALK-negative ALCL, clustering together and separate from all other types of investigated NHLs and NS-cHL in particular, would have similar reactive infiltrates in their stromal component. This would also explain why PMBCL, ABC-DLBCL, and GC-DLBCL, all of which have a less pronounced number of non-tumour bystander cells, cluster separately from NS-cHL, ALCL-like HL, and ALK-negative ALCL.

The acquired dendrogram additionally suggests that ABC-DLBCL is more related to PMBCL than to GC-DLBCL. This association might be at least partially explained by their comparable constitutive activation of the NF-κB pathway as a major source of tumour cell survival [30,41]. Although NF-κB is also activated in cHL, the striking presence of regions coding for genes involved in this pathway in the CESH profiles, comparing PMBCL and ABC-DLBCL with NS-cHL, might be justified by the higher percentage of tumour cells in these lymphomas compared with NS-cHL. Some examples of these genes include *F2R* (5q13), *ICE* and *c-IAP2* (11q22), *MALT1* (18q21), and *BTK* (Xq22), which are upstream activators of NF-κB [42–44], and *BIRC2* and *BIRC3* (11q22), *BCL2* (18q21), and *XIAP* (Xq25), which represent NF-κB target genes with anti-apoptotic properties [45,46].

CESH profiling of all investigated lymphomas versus reactive lymph nodes confirmed the initial results obtained pairing the NHLs with NS-cHL. The foremost conclusion from profiling and unsupervised hierarchical clustering of the individual cases as well as the pooled samples is that the acquired CESH profiles are representative for the investigated lymphoma entities. After all, each pooled sample clustered with its respective individual cases. Moreover, the acquired dendrogram supports the idea that cHL is a disease separate from the NHLs. Consequently, the morphological grey zone between NS-cHL and the investigated NHLs is not reflected in the molecular genetic data. Finally, we demonstrated that ALK-negative ALCL clustered together with ALCL-like HL. Respectively, 50% and 76% of the relative CESH profiles pairing ALK-negative ALCL and ALCL-like HL with NS-cHL form a common signature, supporting the presence of a spectrum between ALCL-like HL and ALK-negative ALCL, distinguishing them from NS-cHL. Genes encoded in chromosomal regions differentially expressed between NS-cHL and ALCL-like HL may offer a molecular genetic basis for new clues to improve their differential diagnosis and therapeutic approaches.

In summary, we have established a unique CESH expression profile for all investigated lymphoma types that were defined by conventional morphological criteria. Unsupervised hierarchical cluster analysis of our data showed that NS-cHL is a disease distinct from all NHLs included in this study, including ALCL-like HL. Moreover, analysing the neoplastic cells together with their reactive microenvironment showed that ALK-negative ALCL and ALCL-like HL seem to be related to each other, suggesting a particular role for the stromal component in their expression profiles.

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Supplementary material

Supplementary material may be found at the web address http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2043.html

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