Indications for peripheral light-chain revision and somatic hypermutation without a functional B-cell receptor in precursors of a composite diffuse large B-cell and Hodgkin's lymphoma

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Composite lymphomas are rare combinations of Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma in the same patient, where clonal relatedness has been observed in most of the few cases analyzed. Here, we report a composite classical HL and diffuse large B-cell lymphoma (DLBCL) with interesting molecular features. Micromanipulation of single cells and analysis of V gene rearrangements revealed clonal relatedness with shared and distinct mutations, indicative of derivation from a common germinal center (GC) B-cell precursor and also of further development of both lymphomas in a GC. In the DLBCL, a very high mutation load, including inactivating mutations, and two copies of the same clonal rearrangement with different mutations in single cells were observed. Intriguingly, in the DLBCL precursor somatic hypermutation activity continued after acquisition of destructive V gene mutations, a feature previously found only in Epstein–Barr virus (EBV) infected B-cell expansions. Furthermore, we found evidence of light-chain receptor revision in the lymphoma precursor during a GC reaction. Re-expression of the V(D)J recombination machinery may enhance genomic instability in GC B cells and contribute to lymphomagenesis.

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Traditionally, malignant lymphomas have been divided into two distinct groups, Hodgkin's lymphomas (HL) and non-Hodgkin's lymphomas (NHL). The majority of NHL have been shown to derive from either B or T cells,¹ whereas the origin of the tumor cells in HL has remained unknown for many years. However, using micromanipulation of single cells and molecular analysis of the immunoglobulin (Ig) genes in HL, several reports revealed that the Hodgkin's and Reed–Sternberg (HRS) cells in most cases represent clonal populations of transformed B

cells.^{2–4} In rare patients, occurrence of both NHL and HL is observed, either sequentially or simultaneously, and it is an interesting issue whether such composite lymphomas are clonally related or if occurrence is only coincidental or therapy-related, especially if there is a long time span between the diagnoses.

Each mature B cell carries rearranged V genes encoding the heavy and light chains of the B-cell receptor (BCR).^{5,6} During somatic recombination of the V, (D), and J segments, extensive junctional diversity is created, making V gene rearrangements ideal 'fingerprints' for clonality analyses.⁷ Furthermore, as V gene rearrangements are modified in germinal center (GC) reactions by somatic hypermutation, analysis of somatic mutation patterns allows assignment of B-cell lymphomas to various maturation stages of B cells.^{6,8,9} The recent application of

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such analyses to a few cases of composite lymphomas showed that in most instances common lymphoma precursors existed.^{10–15} For example, composite lymphomas consisting of follicular lymphoma and HL, or diffuse large B-cell lymphoma (DLBCL) and HL were reported to have clonally related V gene rearrangements.^{10,12} Interestingly, the Ig rearrangements in these cases showed both shared mutations and mutations that were present only in the tumor cells of either of the lymphomas. This pattern of shared as well as distinct mutations in the clonally related lymphomas suggested that the common precursor of the two lymphomas was a GC B cell.

In this study, we analyzed a composite lymphoma with a combination of DLBCL and HL in the same lymph node. A common derivation was shown since clonally related Ig gene rearrangements were amplified from single DLBCL and HRS cells, but the Ig gene rearrangements were further diversified in the distinct lymphomas during tumor development.

Materials and methods

Patient Material

A 74-year-old woman was referred to Department of Internal Medicine, Verona University, for abdominal pain in May 2002. A computed tomography scan revealed a solid retroperitoneal mass (9 × 9 × 12 cm) infiltrating aorta, inferior vena cava and psoas muscle. Numerous enlarged abdominal lymph nodes were also detected. A fine-needle aspiration of the mass showed a cytology consistent with a DLBCL with frequent multilobated morphology. A large lymph node was palpable in inguinal right region and surgically removed; no other superficial lymph nodes were detected. Histological examination of the inguinal lymph node biopsy revealed a composite lymphoma with DLBCL and classical HL of mixed cellularity type. A bilateral bone marrow biopsy was additionally performed, showing involvement of HL, whereas no evidence of DLBCL infiltration was found. The patient was considered to be in stage IVB. After six cycles of rituximab-CHOP (cyclophosphamide, doxorubicine, vincristine, prednisone), the patient responded well with subtotal regression of the lymphoid masses and good peripheral blood count. At 12 months from diagnosis, the patient's general clinical condition appeared good.

Immunohistochemistry, EBER *In Situ* Hybridization and Micromanipulation

Sections of formalin-fixed, paraffin-embedded material were deparaffinized and stained with antibodies for CD30, CD15, CD20, BOB1, and Oct2 using standard procedures. *In situ* hybridization analysis for EBV-encoded RNA (EBER) was performed as previously described.¹⁶ For micromanipulation,

5- μ m-thick frozen tissue sections were stained with anti-CD20 (L26, Dako, Hamburg, Germany) or anti-CD30 monoclonal antibodies (BerH2, Dako) and visualized using the avidin-biotin complex (ABC) technique. Single CD30⁺ HRS cells were micromanipulated with a hydraulic micromanipulator as described earlier.¹⁷ For CD20⁺ DLBCL cells, single cells as well as groups of cells (2–4 cells) were micromanipulated. Isolated cells were stored at –20°C in 20 μ l of 1 × Expand buffer without MgCl₂ (Roche Diagnostics, Mannheim, Germany). For each set of 10 micromanipulated cells or set of 10 clusters of cells, four aliquots of buffer covering the sections were aspirated and used as negative controls in the PCR.

Single-cell PCR Analysis

Single cells or groups of cells were digested with 0.5 mg/ml proteinase K (Roche Diagnostics, Mannheim, Germany) in 1 × Expand buffer for 2 h at 50°C, which was followed by enzyme inactivation at 95°C for 10 min. Amplification of the rearranged Ig genes was performed in a seminested approach, using family-specific V_H leader primers or framework region (FR)I family-specific V_H, V _{κ} and V _{λ} primers together with two sets of J primers designed for each locus as previously described.^{18–20} Amplification of D_H–J_H rearrangements was performed using D_H family-specific primers together with J_H primers as detailed earlier.²¹ This PCR also detects germline configuration of the IgH locus. For amplification of rearrangements between a recombination signal sequence in the J _{κ} –C _{κ} intron (J _{κ} –C _{κ} intron-RSS) region and the kappa-deleting element (KDE) and/or germline configuration of the KDE, one J _{κ} C _{κ} primer, one C _{κ} primer and one first and second round KDE primer were used as described previously.²² PCR products were gel-purified and directly sequenced on an automated sequencer (ABI 3100, Applied Biosystems, Weiterstadt, Germany) using the Big Dye Terminator cycle sequencing kit. Sequences were aligned to Ig sequences from the GenBank and IMGT databases.²³

Results

Histology and Immunohistology

Histological examination of the inguinal lymph node, which measured 5 cm in maximal dimension, showed effacement of the normal structure by two distinct patterns of infiltration. In one area, there was a typical mixed cellular infiltrate of mixed cellularity HL with few HRS cells intermingled. In the same lymph node, separated from the HL, a more homogeneous infiltrate of large-sized B-cell blasts of a DLBCL was observed (Figure 1). Immunohistochemistry demonstrated that the HRS cells were CD30⁺, CD15^{+/–}, and CD20[–], whereas the DLBCL

Figure 1 Histology and immunohistology of a composite HL and DLBCL. (a, b, c) Overviews ($\times 5$ magnification) of the composite lymphoma, in (a) a hemalaun/eosin (HE), in (b) an anti-CD20 and in (c) an anti-CD3 immunostaining. In the three pictures, the DLBCL is in the upper part and the lower right part, whereas the HL is in the lower left part; (d) $\times 200$ magnification of a HE staining from the HL part. Two HRS cells are marked by arrows; (e) $\times 200$ magnification from a HE staining of the DLBCL; (f) anti-CD30 staining of an HRS cell ($\times 400$); (g) anti-CD15 staining of an HRS cell ($\times 400$); (h) anti-CD20 immunostaining of the DLBCL part ($\times 200$); and (i) of the HL part with a CD20-negative HRS cell ($\times 400$).

cells were CD30⁻, CD15⁻, and CD20⁺ (Figure 1). Furthermore, the DLBCL cells expressed BOB1 and Oct2, whereas these transcription factors were not expressed in the HRS cells (not shown). *In situ* hybridization for the small noncoding RNAs of EBV revealed that neither the HRS nor the DLBCL cells were EBV-infected (not shown).

Sequence Analysis of Ig Gene Rearrangements in DLBCL and HRS Cells

Single CD30⁺ HRS cells and groups of 2–4 CD20⁺ DLBCL cells were isolated from frozen tissue sections of the composite lymphoma using micro-manipulation. Cells were then subjected to semi-nested PCR for V_H, V _{κ} and V _{λ} gene rearrangements

using FRI primers and different sets of J gene segment primers. A clonal V_H3–23/J_H6 rearrangement (the D_H gene could not be identified) was amplified from 14 of 20 CD30⁺ cells, whereas no V_H gene rearrangement was obtained from any of the 20 samples with CD20⁺ cells (Table 1). The same clonal V _{κ} 1D–39/J _{κ} 2 rearrangement was amplified from the DLBCL cells (10 of 20 samples) and the single HRS cells (six of 20 cells). Additionally, a second V _{κ} /J _{κ} rearrangement (V _{κ} 1D–6/J _{κ} 2) was obtained from 14 of 20 CD30⁺ cells, whereas none of the CD20⁺ cells displayed this rearrangement (Table 1). Only two V _{λ} /J _{λ} PCR products were amplified, one from a CD30⁺ cell and one from a CD20⁺ cell, indicating that the lymphoma cells do not carry clonal V _{λ} rearrangements (Table 1).

Table 1 Sequence analysis of V gene rearrangements amplified from DLBCL and HRS cells of a composite lymphoma

<i>Cells</i>	<i>Cells positive in PCR</i>	<i>PCR products amplified repeatedly</i>	<i>Original rearrangement potentially functional</i>	<i>Mutation frequency (%)</i>	<i>Functionality after somatic hypermutation</i>	<i>Ongoing mutation</i>
DLBCL						
CD20 ⁺ (2–4 cells/tube)	V _H L: 14/20 V _H FRI ^c : 0/20	14 × V _H 3–23 ^a	+	27–28 ^b	–	No
	V _κ : 10/20 V _λ : 1/20 ^d D _H : 7/10 KDE: 18/20	10 × V _κ 1D–39 7 × unrearranged 18 × unrearranged ^e	+	18	+	No
Negative controls ^f	V _H L, KDE, D _H : 0/4 V _H L, KDE: 0/4 V _H FRI, V _κ , V _λ : 0/8					
CD20 ⁺ (1 cell/tube)	V _H L: 18/42 V _H L: 0/18	18 × V _H 3–23	+	27–28 ^b	–	No
Negative controls ^f						
HL						
CD30 ⁺ (1 cell/tube)	V _H L: 8/16 V _H FRI: 14/20 V _κ : 15/20 ^g	8 × V _H 3–23 14 × V _H 3–23 6 × V _κ 1D–39 ^h 14 × V _κ 1D–6	+	7	+	No
	V _λ : 1/20 ^d D _H : 4/10	4 × unrearranged	+	7	+	No
	KDE: 13/16	10 × unrearranged 11 × J _κ –C _κ intron–RSS/ KDE rearrangement	+	7	+	No
			+	8	+	No
Negative controls ^f	V _H L, KDE, D _H : 0/4 V _H L, KDE: 0/2 V _H FRI, V _κ , V _λ : 2/8 ⁱ					

All sequences were deposited in the EMBL database under accession nos. AJ586890–896.

^aFrom two tubes in addition to the clonal rearrangement, unrelated unique rearrangements (V_H2–26 and V_H4–34) were amplified, likely due to the occasional micromanipulation of nontumor B cells from the DLBCL region.

^bIncluding one 1-bp insertion and three deletions (two 1-bp deletions and one larger deletion of 56 bp).

^cV_HFRI PCR was negative in DLBCL cells due to a large deletion encompassing the primer binding site in FRI.

^dOne V_λ1 PCR product out of 20 HRS cells and one V_λ3 PCR product out of 20 DLBCL cells were amplified but not sequenced. These amplicates likely represent cellular contamination, or picking of a bystander B cell in case of the analysis of CD20⁺ B cells.

^eOne single J_κ–C_κ intron–RSS/KDE rearrangement was amplified from a tube from which also a unique V_H rearrangement was amplified (see footnote a) and is thus likely derived from a nontumor B cell.

^fFor each 10 cells four aliquots of buffer covering the sections during micromanipulation were used as negative controls.

^gFrom two tubes in addition to the clonal rearrangement, unrelated unique rearrangements (V_κ1D–33 and V_κ1D–39) were amplified.

^hFive of six cells have both one V_κ1D–39 and one V_κ1D–6 rearrangement.

ⁱV_κ1D–33 and V_κ1–8 rearrangements not related to the tumor clone.

Amplification was performed on a second set of isolated HRS and DLBCL cells using V_H leader region primers and J_H primers, which then revealed presence of the clonal V_H3–23/J_H6 rearrangement in both cell types: eight of 16 single CD30⁺ cells and 14 of 20 samples with CD20⁺ cells harbored this rearrangement (Table 1). The clonal V_H3–23 rearrangement in the DLBCL component had a large deletion within FRI encompassing the primer binding site (Figure 2a), explaining why the initial V_HFRI PCR was unsuccessful for the DLBCL cells. All clonal rearrangements obtained from HRS and DLBCL cells were in-frame and originally potentially functional (Table 1).

To analyze if the second IgH allele of the DLBCL and HRS cell clones contained D_H/J_H joints or were in germline configuration, we performed PCR with family-specific D_H primers together with J_H primers. For both parts of the composite

lymphoma, only PCR products indicative of germline configuration of the second IgH allele were obtained (Table 1).

Analysis of Somatic Hypermutation Pattern in Rearranged V Genes

The V_H and V_κ rearrangements of the DLBCL and HRS cells were all somatically mutated, but the mutation frequencies were higher in DLBCL than HRS cells: 27–28 vs 7% for the V_H3–23/J_H6 rearrangement and 18 vs 7% for the V_κ1D–39/J_κ2 joint. The V_H and V_κ rearrangements showed mutations shared by the DLBCL and HRS cells (13 V_H and 14 V_κ gene mutations in common) as well as a substantial number of mutations present only in the DLBCL or HRS cell clones (86 and 18 distinct mutations in the V_H3–23/J_H6 rearrangement in DLBCL and HRS cells, respectively, and 33 and eight distinct mutations in

Figure 2 Nucleotide sequences of the V_H3-23 gene rearrangement (**a**) and the $V_{\kappa}1D-39$ gene rearrangement (**b**) amplified from the DLBCL (sequence variants I and II) and HRS cells. Sequences were aligned to the most homologous germline genes. The coding sequence is shown in triplets. Dots indicate sequence homology and dashes, missing base pairs. The intron region as well as the CDRs are indicated. Shared mutations and the FRI V_H3 primer binding site are highlighted. Stop codons are marked with asterisks. HL, Hodgkin's lymphoma; DLBCL, diffuse large B-cell lymphoma.

the $V_{\kappa}1D-39/J_{\kappa}2$ rearrangement in DLBCL and HRS cells, respectively) (Figures 2 and 3).

Assignment of the amplified sequences to germline V genes was unequivocal for the HRS cells (V_H3-23 rearrangement: 31 mutations compared to V_H3-23 but 43 mutations compared to V_H3-66 , the next homologous germline gene; $V_{\kappa}1D-39$: 22 mutations compared to $V_{\kappa}1D-39$ but 36 mutations compared to $V_{\kappa}1-6$ and $V_{\kappa}1-16$, the next homologous germline genes) and also for the DLBCL $V_{\kappa}1D-39$ rearrangement (49 mutations compared to $V_{\kappa}1D-39$ but at least 56 and 57 mutations compared to $V_{\kappa}1-6$ and $V_{\kappa}1-16$, the next homologous germline genes). The two variants (see below) of the V_H rearrangement amplified from the DLBCL had the same number of differences compared to V_H3-23 and V_H3-53 (variant I, 101 differences) and V_H3-23 and V_H3-66 (variant II, 105 differences) germline V

genes. However, the clonal relatedness of the HRS and DLBCL cells, based on similarities of the V_H CDRIII and $V_{\kappa}1D-39$ rearrangements, together with the unequivocal assignment of the HRS cell V_H rearrangement to V_H3-23 and several shared mutations between the HRS and DLBCL V_H rearrangements, strongly argue that also both DLBCL V_H variants use the V_H3-23 germline gene.

In contrast to the HRS cells, the V_H gene rearrangement of the DLBCL cells carried several inactivating mutations in the V_H gene: two stop codons and one large deletion of 56 bp (Figure 2). The $V_{\kappa}1D-6$ rearrangement, which was amplified only from HRS cells, was also somatically mutated, with a mutation frequency of 8%.

The rearrangements of the HRS cells did not show any intraclonal diversity among the 36 cells analyzed. However, sequence analysis of the V_H and

Figure 3 Genealogical tree of the clonal development in this composite lymphoma. Two hypothetical common ancestors (I and II) have been indicated. The number of shared or additional mutations in the different clones is given. KDE, kappa-deleting element; KDE unrearr., germline configuration of the KDE; JKDE rearr., J_{κ} - C_{κ} intron-RSS/KDE rearrangement.

V_{κ} rearrangements of the DLBCL cell samples revealed 'double peaks' in the electropherograms, indicating mixed sequences, on eight positions for the V_H gene rearrangement and three positions for the V_{κ} gene rearrangement (Figure 2). Since 2–4 DLBCL cells were analyzed together in single tubes, the sequence variations could be due to presence of two DLBCL subclones with distinct mutation patterns, or to presence of different mutated copies of the V gene rearrangements within single cells. To clarify this issue, we performed IgH analysis on 42 isolated single DLBCL cells (Table 1). From 13 cells, sequences without 'double peaks' were obtained, representing two distinct mutation patterns: five of these sequences showed the same two additional mutations in addition to the mutations shared by all DLBCL cells (sequence type DLBCL I), and eight cells carried six additional mutations (DLBCL II) (Figure 2a). Importantly, from another five single cells a mixture of both sequence variants was amplified. In addition, one cell showed a 'double peak' at a unique position. The amplification of mixed sequences from a considerable fraction of single cells indicates that the DLBCL cells harbor two differentially mutated V_H 3–23 rearrangements

(that mixed sequences were not obtained from all cells is most likely due to the fact that the PCR efficiency for single micromanipulated cells is usually below 50%).¹⁸ Moreover, there is no significant ongoing mutation within the DLBCL clone.

Peripheral Receptor Revision in the Composite DLBCL/HL

Since we amplified one clonal V_{κ}/J_{κ} joint from both types of tumor cells of the composite lymphoma whereas a second V_{κ}/J_{κ} joint could be amplified only from the HRS cells, we further characterized the Ig κ loci using a PCR to detect J_{κ} - C_{κ} intron-RSS/KDE rearrangements and also KDE germline configuration (Figure 4).²² The KDE is located 3' of the C_{κ} gene, and by rearrangement of the KDE to an unrearranged V_{κ} gene segment or to an RSS site in the J_{κ} - C_{κ} intron, the intervening region including the C_{κ} gene and both κ enhancers is deleted.^{24,25} Such rearrangements are mainly used to inactivate the Ig κ loci in lambda-expressing cells. The PCR for KDE rearrangements further substantiates that there are likely different rearrangements at the Ig κ loci in both entities of the composite lymphoma: from the HRS cells a clonal J_{κ} - C_{κ} intron-RSS/KDE rearrangement was amplified (11 of 16 cells) in addition to a PCR product indicative of germline configuration of the KDE in the second Ig κ allele (10 of 16 cells), while from the DLBCL cells only PCR products showing germline configuration were obtained (18 of 20 cells) (Table 1). We could not determine if the germline KDE PCR products from the DLBCL were derived from two or only one Ig κ allele, since we did not detect any polymorphisms in the region amplified with the KDE primers using DNA from whole tissue sections.

The combinations of PCR products amplified from the two parts of the composite lymphoma—clonal $V_{\kappa}1D-39/J_{\kappa}2$, $V_{\kappa}1D-6/J_{\kappa}2$ and J_{κ} - C_{κ} intron-RSS/KDE rearrangements and KDE germline configuration from the HRS cells, but only the clonal $V_{\kappa}1D-39/J_{\kappa}2$ and KDE germline configuration from the DLBCL cells—can be explained at least in two ways. One hypothesis is that the precursor of both lymphomas contained only the $V_{\kappa}1D-39/J_{\kappa}2$ joint and, by receptor revision, the $V_{\kappa}1D-6/J_{\kappa}2$ joint was rearranged in the HRS precursor. Since the HRS and DLBCL clone share somatic V gene mutations, and since the J_{κ} - C_{κ} intron-RSS/KDE rearrangement abolishes somatic hypermutation on the respective Ig κ allele (due to concomitant deletion of the κ enhancers that are needed for hypermutation),²⁶ the $V_{\kappa}1D-6/J_{\kappa}2$ rearrangement and the J_{κ} - C_{κ} intron-RSS/KDE rearrangement likely happened after acquisition of mutations in the $V_{\kappa}1D-39/J_{\kappa}2$ joint, that is, in a GC reaction. Alternatively, both V_{κ}/J_{κ} joints may have been present in a common precursor of the two lymphomas, but the allele harboring the $V_{\kappa}1D-6/J_{\kappa}2$ joint was later lost in the DLBCL clone (eg by chromosomal deletion or V_{κ} /KDE

Figure 4 Amplification of rearrangements involving the KDE. The upper part of the scheme shows an Ig κ locus with a V κ /J κ joint, the J κ intron RSS, both κ enhancers, the κ constant region and the KDE. Positions of primers used to detect amplificates involving the KDE are indicated below. Rearrangements involving the KDE as well as the resulting PCR products are shown in the middle of the scheme.

rearrangement). Importantly, also for this scenario, the KDE rearrangement in the HRS cells should have happened after mutations accumulated in the V κ /J κ joints, that is, in the GC, as argued above. Hence, the presence of two mutated V κ /J κ joints, both using J κ 2, together with a J κ -C κ intron-RSS/KDE rearrangement, clearly argues that Ig κ rearrangement(s) occurred during a GC reaction in the HL precursors.

Discussion

Several composite lymphomas of HL and NHL have recently been investigated using molecular techniques and a clonal relation has been found in most of these cases.^{10–15} In this study, we investigated a composite lymphoma of HL and DLBCL and were able to show clonally related V_H and V κ gene rearrangements in both lymphomas, which reveals derivation from a common lymphoma precursor also in this case. The amplified Ig gene rearrangements were somatically mutated in both lymphomas, showing shared as well as distinct mutations. This combination of shared as well as distinct mutations strongly suggests that the two lymphomas derive from two distinct members of a common GC B-cell clone. Presumably, the common shared precursor already carried some transforming event(s), and further distinct genetic lesions were acquired by the precursors of the DLBCL and HL, likely also in the GC microenvironment (Figure 3).

Analyses of Ig rearrangements in B-cell lymphomas have revealed specific features for several entities and were instrumental to assign the lymphomas to various maturation stages of normal B cells.^{7–9} Thus, HRS cells of classical HL are

considered to derive from preapoptotic GC B cells with mutated V gene rearrangements and obviously crippling mutations in a considerable fraction of cases but without ongoing somatic hypermutation,³ while DLBCL usually carry functional V gene rearrangements with or without intraclonal sequence diversity.^{27,28} In the present case, intraclonal diversity was neither observed in the HRS nor in the DLBCL cells. However, the DLBCL cells carried two copies of the V_H rearrangement with a distinct mutation pattern (Figure 2). This finding is most likely due to duplication of the IgH locus carrying the rearrangement and continued hypermutation after the duplication event. A similar observation was previously made in a single-cell analysis of T-cell-rich B-cell lymphomas, a subtype of DLBCL, and presence of duplications was there confirmed by FISH analysis.²⁰ The DLBCL in the present case carried a much higher mutation load than the HRS cells, indicating that the hypermutation process was active for a longer time and more rounds of mutation in the DLBCL than in the HRS cell precursor before it was finally silenced.

Somewhat surprisingly, the DLBCL contained several crippling mutations in the V_H rearrangement, whereas the HRS cells carried no obviously destructive mutations. As the second IgH alleles are in germline configuration in the lymphomas, the DLBCL could not express a functional BCR. While inactivating mutations are frequently found in HRS cells of classical HL, they are relatively rare in B-NHL.^{7,29} Expression of a BCR is mandatory for normal B cell survival, and presumably also generates important survival signals for most B-cell lymphomas.^{8,30} Besides classical HL, BCR-deficient B-cell clones have been found mainly in

post-transplant lymphomas and in B-cell clones expanding in T-cell lymphoma of angioimmunoblastic dysproteinemia type.^{31–33} In these diseases, the B-cell clones are usually EBV-infected, arguing for a role of the virus (in particular LMP2a expression) in replacing the survival signal normally mediated by the BCR. Notably, the inactivating mutations in the V_H rearrangement of the DLBCL (see above) were shared between both sequence variants. Consequently, the distinct mutations must have been acquired after loss of the capability to express a functional BCR, showing that hypermutation activity continued for some time after acquisition of the crippling mutations, before it was silenced.

An intriguing feature observed in this composite lymphoma are the indications for peripheral light-chain revision during lymphoma development. Light-chain replacement has been shown to take place in early B-cell development in order to rescue B cells with autoreactive properties from apoptosis by acquisition of a new V_L gene rearrangement.^{34,35} Over the last years, several reports indicated that receptor revision may also occur in peripheral lymphoid organs in GC reactions.^{36–44} The maturation stage of the revising cells remains, however, controversial,^{45–51} and an analysis of single peripheral B cells of healthy humans indicated that receptor revision in GC reactions might not contribute significantly to the diversification of the memory B-cell pool.²² A few reports observed traces for receptor revision also in B-cell malignancies, either involving replacement of V_H genes or novel rearrangement in the light chain loci.^{13,20,52} In our case, we observed a configuration of the Igκ loci which, together with the mutation pattern in the V_κ/J_κ joints, indicates that light chain revision occurred during a GC reaction in a precursor of the HRS cells. If the V gene recombination machinery can indeed be reactivated in GC B cells, mistargeting of this process may sometimes contribute to lymphoma pathogenesis, for example, by causing chromosomal translocation.⁵³

Taken together, molecular investigation of this composite lymphoma of HL and DLBCL suggests a common GC clone derivation as has been shown previously for other combinations of HL and B-cell NHL. However, in this composite lymphoma the NHL carried a crippled V_H rearrangement and sustained somatic hypermutation occurred in an already BCR-less precursor. In addition, the indications for light-chain receptor revision in a GC reaction imply that activation of the V(D)J recombination machinery may enhance genomic instability in GC and also contribute to lymphomagenesis.

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