Chromosomal Breakpoints Affecting Immunoglobulin Loci Are Recurrent in Hodgkin and Reed-Sternberg Cells of Classical Hodgkin Lymphoma

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Abstract

Chromosomal breakpoints affecting immunoglobulin (IG) loci are recurrent in many subtypes of B-cell lymphomas. However, despite the predominant B-cell origin of the Hodgkin and Reed-Sternberg (HRS) cells in classical Hodgkin lymphoma (cHL), the presence of chromosomal translocations in IG loci has not yet been systematically explored. Therefore, we have investigated a series of cHL for chromosomal breakpoints in the IGH (n = 230), IGL (n = 139), and IGK (n = 138) loci by interphase cytogenetics. Breakpoints in the IGH, IGL, or IGK locus were observed in the HRS cells of 26 of 149 (17%), 2 of 70, and 1 of 77 evaluable cHLs, respectively. The IG partners could be identified in eight cHLs and involved chromosomal bands 2p16 (REL), 3q27 (BCL6, two cases), 8q24.1 (MYC), 14q24.3, 16p13.1, 17q12, and 19q13.2 (BCL3/RELB). In 65 of 85 (76%) cHLs evaluable for an IGH triple-color probe, the HRS cells showed evidence for a (partial) deletion of the IGH constant region, suggesting the presence of class switch recombination (CSR). Furthermore, analyses with this probe in cases with IGH breakpoints indicated that at least part of them seem to be derived from CSR defects. Our results show that chromosomal breakpoints affecting the IG loci are recurrent in cHL.

(Cancer Res 2006; 66(21): 10332-8)

Introduction

In contrast to other B-cell malignancies, the pattern of chromosomal aberrations in classical Hodgkin lymphoma (cHL) is poorly characterized (1, 2). On the one hand, this is due to the difficulty of obtaining metaphases from the rare Hodgkin and Reed-Sternberg (HRS) cells, which account for only 0.1% to 1% of the cells in the tumor biopsies. On the other hand, metaphases from HRS cells show an extreme cytogenetic complexity (3, 4). Molecular cytogenetic techniques specifically targeting the HRS cells have recently provided first insights into cytogenetic aberrations in the HRS cells. These techniques include comparative genomic hybridization (CGH) from microdissected HRS cells, fluorescence in situ hybridization (FISH), and fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasms (FICTION). These studies reported genomic gains in chromosomal arm 2p, including the REL gene, as well as gains in 9p to be present in 30% to 50% of cHLs (4–7). However, recurrent chromosomal translocations have not yet been characterized in cHL.

In many B-cell lymphomas, chromosomal translocations affecting the immunoglobulin heavy chain (IGH) locus in 14q32.3 are highly recurrent. Variants of these aberrations involve the light chain κ (IGK) in 2p11.2 or λ (IGL) in 22q11.2. As a result of these translocations, oncogenes are placed under the control of IG regulatory elements leading to deregulated expression (8, 9). Some IG translocations are closely associated with certain lymphoma subtypes. The t(8;14)(q24.1;q32.3) (MYC/IGH) is the hallmark of Burkitt lymphoma (BL; ref. 10), and the t(11;14)(q13.2;q32.3) (CCND1/IGH) is present in 90% of mantle cell lymphomas (MCL; ref. 11). In cHL, in spite of the germinal center B-cell origin of most HRS cells, the presence of chromosomal breakpoints in IG loci has not been systematically explored thus far. Cytogenetic studies in cHL have reported the presence of breakpoints affecting band 14q32 (containing the IGH locus) in 10% to 20% of the cases (12–14). With regard to specific translocations, the only published data refer to the lack of t(14;18)(q32.3;q21.3) (IGH/BCL2 fusion) in microdissected HRS cells of cHL patients (15). However, a single cHL case has been reported by FISH to carry an IGH/BCL2 fusion (16), and HRS cells from composite lymphomas have been shown...
to carry translocations of \textit{IGH} to \textit{CCND1} (MCL and cHL) or \textit{BCL2} \citep{folllicular lymphoma (FL) and cHL; refs. 17, 18}. Translocations involving \textit{BCL6} have been reported to be absent in cHL, whereas they are recurrently present in nodular lymphocyte predominant Hodgkin lymphoma (NLPHL; ref. 19). Other oncogenes involved in \textit{IG} and non-\textit{IG} translocations in B-cell lymphomas, such as \textit{MYC} and \textit{MALT1}, have not yet been systematically studied in cHL \citep{2}.

Chromosomal translocations affecting \textit{IGH} are thought to arise as by-products of incorrect V(D)J recombination, somatic hypermutation, or class switch recombination (CSR; refs. 8, 20). In cell lines from cHL, the \textit{IGH} locus in HRS cells was shown to undergo CSR, which is typical for germinal center–experienced B cells \citep{21}. However, studies on CSR in primary cHL are hampered by difficulties of analyzing CSR in single HRS cells.

Here, we have applied interphase cytogenetic techniques (FISH and FICTION) to a series of cHL to investigate the presence chromosomal breakpoints affecting the \textit{IG} loci and oncogenes involved in B-cell malignancies, such as \textit{BCL6}, \textit{MYC}, and \textit{MALT1}. Moreover, we studied CSR of the \textit{IGH} locus in primary cHL. Our data show that translocations affecting the \textit{IG} loci are recurrent in cHL, most likely derived from erroneous CSR, and involve different partner genes.

Materials and Methods

\textbf{Patient material}. Tumor samples of 232 patients diagnosed with cHL were derived from German, French, and Russian pathology and cytogenetic institutes. Among them, 167 were cytogenetic suspensions, 44 were cytospin preparations, 19 were cryosections, and 2 were touch preparations. This study has been done within the joint project "Molekulare Mechanismen bei malignen Lymphomen" for which central and local ethics approval was obtained. From 171 cHLs with available conventional cytogenetic analyses, 15\% displayed highly complex hyperploid metaphases. In the remaining cHLs, cytogenetic analysis rendered either normal or no metaphases. The karyotypes of cHL1 and cHL27 have been reported previously \citep{7, 22, 23}.

Paraffin-embedded material from a total of 27 cHLs with FISH- or FICTION-proven chromosomal translocations affecting any of the \textit{IG} loci was centrally reviewed by a panel of expert hematopathologists (Table 1). The diagnosis of cHL was confirmed in 25 cases. The remaining two cases were derived from German, French, and Russian pathology and cytogenetic institutes. Among them, 167 were cytogenetic suspensions, 44 were cytospin preparations, 19 were cryosections, and 2 were touch preparations. This study has been done within the joint project "Molekulare Mechanismen bei malignen Lymphomen" for which central and local ethics approval was obtained. From 171 cHLs with available conventional cytogenetic analyses, 15\% displayed highly complex hyperploid metaphases. In the remaining cHLs, cytogenetic analysis rendered either normal or no metaphases. The karyotypes of cHL1 and cHL27 have been reported previously \citep{7, 22, 23}.

Paraffin-embedded material from a total of 27 cHLs with FISH- or FICTION-proven chromosomal translocations affecting any of the \textit{IG} loci was centrally reviewed by a panel of expert hematopathologists (Table 1). The diagnosis of cHL was confirmed in 25 cases. The remaining two cases originally diagnosed as cHL were reclassified as T-cell-rich B-cell lymphoma and B-cell non–Hodgkin’s lymphoma (B-NHL) not otherwise specified and, thus, removed from the final analysis. Particularly, the pathology review carefully ruled out the possible presence of NLPHL or coexisting B-NHL in the cases with cHL. Three cHLs with IG breaks could not be centrally reviewed. Patient features of the total of 28 cHLs with breakpoints in any of the \textit{IG} loci are shown in Table 1.

\textbf{FISH and FICTION}. The commercially available locus-specific identifier (LSI) \textit{IGH} break-apart FISH probe (centromeric to \textit{IGH} labeled in spectrum orange and \textit{IGH} variable region in spectrum green; Abbott/Vysis, Downers Grove, IL) was applied to detect chromosomal breakpoints in the \textit{IGH} locus. To study the presence of CSR and characterize cases with \textit{IGH} breakpoints, a three-color probe combining the LSI \textit{IGH} assay with a bacterial artificial chromosome (BAC) clone RP11-417P24 (accession number AL122127; labeled with diethyl aminomethyl coumarin) was applied. RP11-417P24 covers part of the D segments, J segments, and constant regions M, D, G3, and G1 of the \textit{IGH} locus. G1/G3 segments show high homology to the more centromeric G2/G4, which are likely to result in cross-hybridization (Fig. 1A; ref. 24). In part of the cases, previously described probes flanking the \textit{IG} and \textit{IGH} loci \citep{25} and the LSI \textit{MYC}, LSI \textit{BCL6}, and LSI \textit{MALT1} break-apart probes (Abbott/Vysis) were applied. FISH was done according to routine protocols \citep{25}. Putative HRS cells were identified by virtue of their larger nuclear size and frequent hyperploid status. Cases with low proportions of diploid HRS cells and small nuclei, if any, could not be identified.

For FICTION studies, a mouse monoclonal antibody to CD30 (DakoCytomation, Hamburg, Germany) detected with a rabbit anti-mouse secondary antibody conjugated with Alexa Fluor 594 (Molecular Probes, Leiden, the Netherlands) was applied as previously described in combination with the FISH probes quoted above for the \textit{IG} loci \citep{26}. HRS cells were identified by virtue of their typical morphology, frequent hyperploid status, and expression of the CD30 antigen.

The cutoff values for \textit{IG} break-apart probes have been described previously \citep{25, 27}. However, the peculiarity of the FISH and FICTION approaches described herein is that they allow a targeted analysis of the putative HRS cells. Therefore, classic cutoff values are not applicable. To solve this issue, small round nuclei by FISH or CD30-negative small cells by FICTION served as internal controls and were compared with the results obtained from HRS cells.

As an attempt to identify the \textit{IGH} translocation partners, the probes LSI \textit{BCL2}/\textit{IGH} or LSI \textit{BCL2} break apart, LSI \textit{CCND1}/\textit{IGH} or LSI \textit{CCND1} break apart, LSI \textit{BCL6}, LSI \textit{MYC}/\textit{IGH} CE Phos, LSI \textit{MYC}, and LSI \textit{MALT1} (Abbott/Vysis) were applied. Moreover, self-developed probes for \textit{BCL6}/\textit{IGH}, \textit{REL}/\textit{BCL11A}, \textit{BCL3}, and \textit{BAX} were used. In three cHLs with \textit{IGH} breakpoint and tumor metaphases available, the partner chromosome was identified and characterized by sequential R-bandning and metaphase FISH using probes located in chromosome arms 1q4, 16p, and 17q. In one of these cHLs, a whole chromosome painting probe for chromosome 14 (AGS GmbH, Heidelberg, Germany) was applied. A description of all FISH probes applied in this study is provided as Supplementary Table S1.

In cHL with \textit{IGH} breakpoint, the \textit{REL}/\textit{BCL11A} and \textit{JAK2} genomic status was determined as the ratio between the median number of \textit{REL}/\textit{BCL11A} and \textit{JAK2} hybridization signals per case, and its estimated ploidy level was calculated as median of number of signals for 10 different loci (data not shown). Ratios between 1.3 and 1.99 were considered as gains, whereas ratios \textgreater{}2 were considered as amplifications.

For both FISH and FICTION, slides were analyzed by use of a Zeiss Axiostar 2 fluorescence microscope (Zeiss, Göttingen, Germany) equipped with appropriate filter sets (AHF, Tübingen, Germany) and documented using the ISIS imaging system (MetaSystems, Altlussheim, Germany). A minimum number of 10 tumor cells per case and probe were scored by FISH or FICTION whenever possible.

Results

\textbf{Molecular cytogenetic analyses for the detection of chromosomal breakpoints affecting the \textit{IG} loci}. For \textit{IGH}, large hyperploid nuclei were detected by FISH in 95 of 165 (58\%), whereas typical CD30-positive cells were detected by FICTION in 54 of 65 (83\%), leading to a total number of 149 of 230 (65\%) evaluable cHLs. From these, 26 (17\%) cHLs displayed signal constellations pointing to chromosomal breakpoints in the \textit{IGH} locus. No differences in the incidence of \textit{IGH} breaks were observed by FISH \citep{18, 19} and FICTION \citep{8, 15, 14}. In particular, a split of the \textit{IGH} flanking probe was observed in 18, loss of the telomeric signal in 3, and loss of the centromeric signal in 5 cases. Considering the hyperploid nature of the HRS cells, signal numbers for telomeric and/or centromeric probes exceeding the diploid status were observed in the great majority of the cases (Fig. 1).

For 41\% of 113 (48\%) could be evaluated by FISH and 16 of 26 (62\%) by FICTION (total 70 of 139, 50\%), whereas for \textit{IGK}, 57 of 112 (51\%) could be evaluated by FISH and 20 of 26 (77\%) by FICTION \citep{total 77 of 138, 56\%}. From these, two (3\%) and one (1\%) cHLs displayed signal constellations indicating chromosomal breakpoints in the \textit{IGL} or \textit{IGK} loci, respectively. Remarkably, \textit{CHL} contained two concurrent \textit{IG} translocations involving \textit{IGK} and \textit{IGH}. Results are shown in Table 1.

A median number of 10 (range, 5-36) nuclei per case meeting the criteria for HRS cells were scored in cHL with \textit{IGH} translocations. In 24 of 28 cHLs, all putative HRS cells scored contained signal constellations pointing to the presence of an \textit{IG} translocation...
Cancer Research 2006; 66: (21). November 1, 2006 10334 www.aacrjournals.org

Table 1. Clinical, immunohistochemical, and molecular cytogenetic data of cHL with chromosomal translocations affecting the IG loci

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NOTE: Clinical information on cases 10, 16 to 20, 23, 24, and 26 was not available. Diagnoses in bold letters indicate that the case has been centrally reviewed.

Abbreviations: M, male; F, female; MC, mixed cellularity; NS, nodular sclerosis; UN, unspecified; CR, complete remission; R, relapse; PD, progressive disease; PR, partial remission; UC, under chemotherapy; NR, no response; ND, not done; NI, not identified; NE, not evaluable.

¹Patient deceased.

¹The diagnoses of these cases were established by an experienced hematopathologist but could not be centrally reviewed.

²In contrast to the remaining cHL studied, cHL27 did not contain clearly identifiable large nuclei. However, this case was HRS cell rich and an aberrant signal constellation was observed in 34% of all scored nuclei.

(Table 1). In three cases (cHL7–cHL9), the percentage of putative HRS cells with IG split ranged from 88% to 96%. In case cHL27, tumor cells could not be identified by their nuclear size but the IGH cells with (Table 1). In three cases (cHL7–cHL9), the percentage of putative HRS cells with IG split ranged from 88% to 96%. In case cHL27, tumor cells could not be identified by their nuclear size but the proportion of HRS cells in this atypical cHL was high enough to detect an IGL split (in 34% of the cells). As internal control, bystander cells regularly displayed an unaltered signal constellation.

The REL genomic status could be determined in 26 of the 28 cHLs with IG breakpoints. Nine and five cHLs had amplifications and gains of the REL locus (54% in total), respectively. In 12 cHLs, imbalances of the REL locus were not detected. The JAK2 genomic status was determined in 14 cHLs with IG breakpoints. Two and four cHLs had amplifications and gains of the JAK2 locus (43% in total), respectively, whereas eight cHLs displayed a balanced JAK2 status. The frequency of REL and JAK2 genomic gains and amplifications was thus similar in cHL with to that without IG chromosomal breakpoints (REL, 54% with versus 48% without IG breaks; JAK2, 43% with versus 40% without IG breaks; refs. 7, 27).¹⁴

Detection of deletions in the IGH constant region and characterization of IGH breakpoints by triple-color FISH. In 85 cHLs, a triple-color assay for IGH was applied. In addition to the IGH break-apart probe, this assay contains a differently labeled BAC clone covering part of the D segments and extending up to the G1 IGH constant region. Cross-hybridization to the G2 and G4 centromeric segments is likely due to sequence homologies

¹⁴Unpublished data.
Translocations Affecting IG Loci in cHL

(1A; ref. 23). In 65 of the 85 (76%) cHLs, the RP11-417P24 signals in the HRS cells were absent or reduced compared with those in the small bystander cells (Fig. 1B). This pattern indicates the presence of deletions affecting part of the IGH constant segments, which in turn strongly suggests that CSR has taken place in these HRS cells. The remaining 20 cHLs did not show any discernible change in the signal intensity of the BAC clone between tumor and bystander cells.

The 86 cHLs in which the triple-color probe was applied included 24 cHLs with IGH breakpoints (17 cHLs with a split of telomeric and centromeric IGH signals, 5 with loss of the centromeric signals, and 2 with loss of the telomeric signals). Eight cHLs did not show any detectable colocalization of IGH centromeric or telomeric signals with RP11-417P24, suggesting the presence of a CSR-derived deletion. In nine cHLs, signals for the IGH centromeric probe colocalized with RP11-417P24 (Fig. 1C), whereas in three cases, the IGH telomeric probe colocalized with RP11-417P24 (Fig. 1D). Finally, four cases displayed a breakpoint within RP11-417P24. These data indicate that the molecular breakpoints associated with translocations of the IGH locus in cHL are heterogeneous.

Study of oncogenes involved in B-NHL as potential IG translocation partners in cHL. To determine whether IG translocations in cHL involve the same partners as other B-cell lymphomas, we investigated the CCND1, BCL2, BCL6, MYC, MALT1, REL/BCL11A, BCL3, and PAX5 loci by FISH/FICTION in the cHL with IG breakpoints (Table 1). Signal patterns indicating the presence of a BCL6 breakpoint were observed in three cHLs (cases 2, 8, and 25). By using a three-color FISH assay, two of these cases were shown to carry a BCL6/IGH fusion (Fig. 1E). Juxtapositions of IGH and MYC (Fig. 1F) or BCL3 as well as of IGL and REL were found in one cHL each (cases 20, 1, and 27, respectively). Hybridization patterns indicating the presence of chromosomal breakpoints in the CCND1, BCL2, MALT1, or PAX5 loci were not observed in any of the remaining cHLs with IG breaks (Table 1).

Characterization of IG chromosomal rearrangements with unknown partners. In three cHLs with IGH breaks, sufficient tumor metaphases were available for further identification of the translocation partner. Metaphase FISH on cHL9 suggested a derivative chromosome 16 to contain the IGH telomeric signal. The breakpoint was narrowed down to a region of 170 kb in 16p13.1 between BAC clones RP11-109M19 and RP11-66H6 (Supplementary Data). This region comprises the MHC2TA and the DEXI genes. A juxtaposition of IGH and BACs RP11-109M19 and RP11-66H6 was further shown by four-color FISH.

In cHL11 and cHL22, R-banding cytogenetic analysis was done followed by FISH with the IGH probe. In cHL11, chromosome band 17q12 was identified as the partner chromosome. FISH proved the break to be located centromeric to the STAT3/STAT5 locus in 17q21.2. In cHL22, R-banding and chromosome painting analyses

![Table 1. Clinical, immunohistochemical, and molecular cytogenetic data of cHL with chromosomal translocations affecting the IG loci](Image)
indicated the presence of a deletion in chromosome 14. This deletion leads to loss of the IGH centromeric signal (Fig. 1G). The centromeric breakpoint of the deletion was located in 14q24.3 and narrowed down to ~100 kb within BAC CTD-3123F15. This clone contains the C14orf43 gene.

Based on the mapping of the novel IGH translocation partners, 13 cHLs in which the chromosomal partner was not identified were screened with probes for 16p13.1 (MHC2TA) and 14q24.3 (C14orf43). None of the cases displayed signal constellations pointing to a chromosomal breakpoint in any of these two loci (Table 1).

Clinical and histopathologic features of cHL with IGH translocations. Fifteen patients with cHL carrying an IGH translocation were female (median age at diagnosis, 23 years; range, 15-73 years) and 13 were male (median age at diagnosis, 34 years; range, 16-74 years). Twenty-one of the 25 panel reviewed cHLs were subtyped as nodular sclerosis (n=15) or mixed cellularity (n=6). Four cases could not be finally subtyped and were classified as unspecified.

All 28 cHLs with IGH breaks studied were CD30 positive. Positivity for CD15 was found in 16 of 24, for CD20 in 2 of 26, for LMP1 in 1 of 19, and for CD3 in none (0 of 22; Table 1).
Clinical data were available from 18 cHLs (Table 1). The median follow-up time was 39 months. Three patients deceased 178, 30, and 4 months after initial diagnosis, and relapses were observed in three cases. These data did not provide evidence for an atypical clinical course in patients with cHL containing IG breaks.

Molecular cytogenetic analyses for the detection of chromosomal changes affecting MYC, BCL6, and MALT1. A total of 42, 46, and 38 cHLs lacking an IG break was evaluated for chromosomal breakpoints affecting the MYC, BCL6, and MALT1 locus, respectively. From this series, one cHL showed a chromosomal breakpoint in the BCL6 locus. The proportion of BCL6 breaks in cHL with IGH translocations (3 of 24, 13%) was higher than that in cHL without IGH translocations 1 of 46 (2%) but did not reach the significance level (P = 0.11, Fisher’s exact test). None of these cHLs displayed breakpoints in MYC or MALT. However, genomic amplifications (>10 signals) of the MYC locus were observed in two cHLs and gains (between 5 and 10 signals) of the MYC, BCL6, and MALT1 loci were observed in five, three, and seven cHLs, respectively.

Discussion

In the present report, we provide evidence that IG translocations are recurrent in cHL. They are present in ~20% of cHLs and, after genomic gains in 2p13-16 and 9p24 (4–7, 28), constitute the third most frequent chromosomal aberration hitherto detected in cHL. Moreover, IGH translocations constitute the first recurrent chromosomal breakpoint in cHL. In line with reports from other B-cell lymphomas (29), IGH breaks occur more frequently than IGL/IGK breaks in cHL (90% versus 10%).

The frequency of EBV infection in cHL is reported to be ~75% in mixed cellularity and 10% to 40% in nodular sclerosis cHL (30). Interestingly, the vast majority of cHL with IG breaks studied herein lacked expression of the LMP1 protein, which was used as surrogate marker for EBV infection (18 of 19) and suggested negativity for EBV. These data might suggest that IG locus-associated chromosomal translocations and infection of HRS cells by EBV represent alternative transforming events in cHL pathogenesis. In contrast, the incidence of chromosomal gains in 2p and 9p in cHL with IG breaks is similar to that in cHL without IG breaks.

It has been previously described that cHL cell lines undergo CSR, which specifically targets the G4 or A1/A2 constant regions (21). Here, using a molecular cytogenetic approach, we provide evidence that at least 76% of the cHLs studied carry a deletion of part of the IGH constant segments identified by BAC RP11-417P24. In FL, the presence of CSR has been detected by array CGH as deletions affecting the very same BAC RP11-417P24 (24, 31). Our findings suggest that CSR has taken place in the majority of cHLs. However, involvement of specific IGH constant genes in cHL could not be determined due to the limitations of the molecular cytogenetic approach.

IGH translocations are thought to derive from errors in VDJ, CSR, or somatic hypermutation processes (8, 20). In cHL, we show IGH translocations to be heterogeneous with regard to chromosomal breakpoints within IGH. In 15 cHLs, hybridization patterns were consistent with a CSR-derived IGH translocation, whereas in 9 cHLs, the breakpoints were more telomeric, and a defect VDJ recombination or somatic hypermutation-associated translocation cannot be excluded.

Contrasting the situation in BL, MCL, and FL but similar to other lymphomas, such as diffuse large B-cell lymphoma or multiple myeloma (2, 8, 9, 32), IG loci seem to be juxtaposed to a wide variety of different partners in cHL. Some of the IG partners in cHL, such as BCL6, MYC, and BCL3, are also recurrently involved in IG translocations in other B-cell lymphomas. Interestingly, BCL6 fusions to IG and non-IG genes are recurrent in NLPHL (19, 33). However, in an independent series of 40 cHLs reported by Wlodarska et al. (19), we could not detect any BCL6 translocations. In the present series, BCL6 breakpoints were observed in 4 of 70 cHLs. Remarkably, BCL6 breaks seemed to be more frequent in cHL with IG translocations than in cHL lacking IG breaks. Our findings also indicate that BCL6 translocations are shared by both cHL and NLPHL and, thus, should not be used as a cytogenetic marker for differential diagnosis.

The molecular cytogenetic characterization of IG translocations in cHL led to the identification of novel potential candidate genes that might be involved in the pathogenesis of cHL. The MHC2TA gene encodes the MHC class II transactivator that has been previously reported to be involved in translocations with BCL6 in B-NHL (34). An additional case displayed a del(14)(q24q32) with a break in IGH and close to the unknown gene C14orf43 in 14q24.3. Homology searches indicated the protein coded by C14orf43 to contain a homeodomain and a Myb DNA-binding domain, which suggests its potential role as transcription factor.

It is widely accepted that IG translocations in B-NHL activate genes placed in the vicinity of IG regulatory elements. In cHL, however, the B-cell signature is lost (35) and the IG transcription machinery is down-regulated by different mechanisms, such as mutations in regulatory regions, lack of B-cell-specific transcription factors, and epigenetic silencing (1, 36, 37). Thus, the consequences of IG translocations in cHL are currently unclear. A tempting hypothesis is that IG translocations in cHL might exert their pathologic function by silencing gene expression of the partner genes. However, this option seems unlikely because part of the IG partner genes in cHL are known as classic oncogenes, such as BCL6, MYC, BCL3, and REL. Published data indicate that the HIS cells in cHL frequently express MYC, BCL3, and c-REL (38–40) but not BCL6 (41). In our series, immunohistochemical studies were done in cHL8 and cHL27 with a BCL6/IGH and REL/IGH translocation, respectively. The former case did not show any detectable expression of the BCL6 protein (data not shown), whereas the latter displayed a high cytoplasmic and nuclear expression of c-REL (38).

Data from studies in composite lymphomas suggest that oncogene deregulation by IG translocations in cHL represents a primary event fostering B-cell transformation. This might be then followed by additional secondary genetic hits that contribute to lymphomagenesis and down-regulate the IG genes. This hypothesis is experimentally supported in composite lymphomas in which, for example, both MCL and cHL shared the translocation t(11;14) (q13.2;q32.3) juxtaposing CCND1 to IGH, but only the MCL cells expressed the cyclin D1 protein (17). Additionally, another composite lymphoma (FL and cHL) sharing a BCL2/IGH translocation showed BCL2 expression from the translocated allele in both FL and cHL fractions (17). This indicates that oncogene deregulation by IG regulatory elements in cHL is possible in some cases regardless of IGH silencing.

There is evidence that chromosomal rearrangements might affect higher-order chromatin structure and, consequently,
gene expression around the breakpoints through changes in chromosome organization within the nucleus (42–44). Thus, an additional hypothesis to explain the possible effect of IG translocations in cHL is that oncogenes juxtaposed to the vicinity of IG might be deregulated by changing their physical location within the nucleus, although this hypothesis deserves further investigation.

In summary, we provide molecular cytogenetic evidence that HRS cells in cHL undergo rearrangements of the IGH locus, which might be derived from CSR. Furthermore, we report that, similarly to other B-cell lymphomas, IG translocations are recurrent chromosomal changes in the HRS cells of cHL. The cloning and characterization of the multiple IG translocation partners in cHL might provide further insights into the pathogenesis and cellular origin of this atypical B-cell lymphoma.

Acknowledgments

Received 5/31/2006; revised 7/24/2006; accepted 8/29/2006.

Grant support: Deutsche Krebshilfe (Network Project "Molecular Mechanisms in Malignant Lymphomas." 70-3173-Ty/31-B).

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We thank Claudia Becker, Dorit Schuster, Magret Ratjen, and Reina Zühlike-Jenisch for their excellent technical assistance.

References

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