Structure and Consequences of IGH Switch Breakpoints in Burkitt Lymphoma

Jeroen E. J. Guikema, Ed Schuuring, Philip M. Kluin

Chromosomal translocations involving the immunoglobulin loci (IGH, IGL, IGK) have been associated with most types of malignant B-cell lymphomas and leukemias. Some of these translocations can be regarded as hallmark characteristics of lymphoma subtypes and are therefore an important part of the diagnosis [e.g., t(11;14) in mantle cell lymphoma and t(14;18) in follicular lymphoma]. Structural analysis has provided insights into the mechanisms leading to these translocations. Because these translocations are generated as an erroneous byproduct of physiological recombination mechanisms that occur in normal B cells, they are instrumental in our understanding of the etiological origin of these lymphomas (reviewed by Kuppers and Dalla-Favera 2001) (1). In Burkitt lymphoma (BL) translocations almost invariably involve the MYC locus at chromosome band 8q24, resulting in the ectopic expression of the c-myc gene, which has a role in cell-cycle regulation and progression. In approximately 75% of the t(8;14)-positive BL tumors, the immunoglobulin heavy-chain locus (IGH) is affected by the translocation, whereas the immunoglobulin light-chain loci (IGL, IGK) are affected in the remainder. DNA sequencing analysis showed that t(8;14) breakpoints are either in the switch (S) regions or in the JH region, implying the involvement of VDJ recombination or somatic hypermutations (SHM) and immunoglobulin class switch recombination (CSR). Although not unequivocally established, current belief is that the JH region breakpoints are generated by an aberrant SHM mechanism (2). Breakpoints in S regions obviously suggest the involvement of CSR. Activation-induced cytidine deaminase (AID) is a B-cell–specific factor that is absolutely required for SHM and CSR (3,4). The t(12;15) translocation found in mouse plasmacytomas is considered as the ortholog of the t(8;14) translocation in humans (5). Using sensitive polymerase chain reaction (PCR) techniques, it has been shown that the t(12;15) translocation in IL-6 transgenic BALB/c mice requires AID, strengthening the point that the t(12;15) is mediated by CSR (6). Another study, however, suggested that the t(12;15) in pristane-induced plasmacytomas in BALB/c mice are generated in an AID-independent fashion, but the development of frank tumors seemed to require AID, implicating AID as a tumor progression factor rather than its involvement in the generation of the initiating translocation (7). The question whether AID is required for the t(8;14) in human BL remains unanswered for obvious reasons and relies on studies done in the mouse. However, from a structural point of view, the t(12;15) in mice is different from the t(8;14) in human BL in several aspects and, importantly, suggests divergent etiological origins for both tumors.

During normal CSR, both Sµ and one of the downstream S regions (Sy, Se, So) are transcribed starting from promoters that are located just upstream of the S regions (8). The base composition of the S regions favors an R-loop structure upon transcription, which is characterized by stretches of single-stranded DNA that is the preferred substrate for AID (9,10). Deamination of cytidines by AID results in uracil residues in the S regions that are subsequently recognized by uracil-DNA-glycosylase (UNG) and processed by the base-excision repair pathway, ultimately resulting in a DNA double-stranded break (DSB) (11,12). How DNA DSBs in Sµ and a downstream S region are synapsed is currently unknown. Synapsis of both S regions might take place before or after induction of DSBs. Synapsed DSBs are resolved by homologous end-joining and the formation of a switch junction, juxtaposing the VDJ coding unit to one of the downstream constant regions, thereby completing the isotype switch. The DNA intervening Sµ and the downstream S regions is ligated to form an extrachromosomal circle, which is eventually lost from the genome. Aberrant CSR leading to a chromosomal translocation involves a third party in this recombination event, typically located on a different chromosome. How and when the interchromosomal interaction takes place is not known, but it has been suggested that the IGH loci and some of the recurrent translocation partner loci are closer to each other in interphase nuclei than
Figure 1. Schematic overview of different configurations of IGH switch translocations. A) Canonical class switch recombination (CSR)–mediated translocation with deletion of Sµ-Sγ3 intervening DNA. B) IGH switch translocation preceded by CSR; the configurations depicted in (A) and (B) are typically found in human multiple myelomas. C) IGH switch translocation followed by CSR; a configuration frequently found in mouse plasma-cytomas. D) Perfect reciprocal IGH switch translocation, as encountered in human Burkitt lymphomas.
would be expected when their nuclear positioning was random (13). A “three-way” translocation involving Sµ, one of the downstream S regions and the putative oncogene present on the translocation partner chromosome results in a configuration in which the translocation breakpoint on the der(14) is in or close to the downstream S region, and Sµ is juxtaposed to the derivative partner chromosome (Figure 1, A). This translocation configuration is usually encountered in human multiple myeloma, which is characterized by IGH S region breakpoints involving various recurrent partner loci (14,15). This configuration could result in the simultaneous dysregulation of two putative oncogenes that are split by the translocation breakpoint. The intrinsic µ enhancer (Eµ), which is located just upstream from Sµ, deregulates an oncogene present on the derivative partner chromosome. The second oncogene, now present on der(14), is affected by the downstream 3′ Ca enhancer (Ea). It has been shown that the MMSET and FGFR3 genes are simultaneously deregulated by such a mechanism in t(4;14)-positive multiple myelomas (16). Additionally, translocation breakpoints downstream of a switch junction have been described in multiple myeloma, which suggests that the translocation event took place after the formation of switch junction and could be the result of an attempted sequential switching activity or possibly a recombination mechanism not involving the CSR machinery (17) (Figure 1, B). In multiple myeloma, the IGH switch breakpoints clearly point toward a germinal center/post-germinal center origin, which is corroborated by the finding that the VDJ genes in multiple myeloma contain SHM without intraclonal variation (18). The configuration of the t(12;15) translocation in spontaneous, and pristane-induced plasmacytomas in Balb/c mice also usually involves a switch–switch junction, but the breakpoints are predominantly located in the 5′ (Sp) part of the junction, suggesting that a CSR event took place after the translocation occurred (19,20) (Figure 1, C). The exact status of the other IGH allele has not been studied in detail, but most mouse plasmacytomas express IgG or IgA, indicating a functional CSR event on this allele. Apparently, the IGH/MYC translocation is not immediately transforming in mouse plasmacytomas as the precursor cells that have acquired the MYC/Sµ breakpoint can continue to undergo CSR and differentiate into plasmablasts/plasmacells, implying a second oncogenic hit at this differentiation stage. A possible explanation for this phenomenon is that the c-myc gene juxtaposed to Sµ is brought closer to the 3′ Ca enhancer by subsequent CSR, resulting in stronger deregulation of c-myc expression.

Noticeably, we observed neither of the above described configurations in BL. Using a DNA fiber–fluorescence in situ hybridization (FISH) technique that allows the assessment of recombination events on both IGH alleles, we showed that the IGH switch breakpoints in BL exclusively involved only one S region instead of two as would be expected from a physiological switch recombination event (Figure 1, D). The IGH allele harboring the translocation breakpoint is not further remodeled by recombination events, as observed in human multiple myelomas and mouse plasmacytomas. Furthermore, the nontranslocated IGH allele did not undergo any form of switch recombination involving deletion of DNA intervening Sµ and a downstream S region (21). However, during normal CSR, generally both alleles undergo recombination. One might question whether CSR is involved at all in this type of translocation and can speculate that the single breakpoint in Sµ or any of the downstream S regions is the result of an intrinsic fragility of S region sequences in germinal center B cells. Human BL tumors have essentially all the prerequisites to undergo CSR, as most tumors express AID or can be induced to do so. This is also true for S region germline transcription (22,23), yet BL almost invariably expresses IgM. This may suggest that BL originates from an early germinal center B cell that is on the brink of undergoing CSR. Moreover, the immunophenotype and gene expression profile support a germinal center derivation for BL (24,25). Also, IgV-genes in BL have somatic mutations, with some degree of intraclonal variation according to several studies (26–28). Altogether, the IGH switch breakpoint configuration clearly indicates that the cell of origin in human BL differs from that in mouse plasmacytomas, which argues against the usefulness of this model to study human BL etiology. Using Eµ-MYC knock-in mice, it has been shown that BL-like tumors can also develop from naïve B cells (29), indicating that the germinal center environment and CSR-related
events are not crucial for tumorigenesis if c-myc expression is appropriately deregulated. However, this model is based upon an a priori engineered translocation and thus not informative about the translocation mechanism.

An interesting consequence of the breakpoint configuration in human BL is that in tumors harboring a breakpoint in one of the downstream S regions, the VDJ-Cµ/Cδ coding unit from the targeted IGH allele is translocated to chromosome band 8q24 in its entirety and could potentially be responsible for IgM expression (Figure 2, A). Previously, we showed by DNA fiber–FISH and long-range PCR that the der(8) chromosome in the Z-138 cell line, which has biallelic IGH translocations, is responsible for IgM expression (30). In addition, we recently demonstrated that this also can occur in primary human BL tumors (21). By DNA fiber-FISH, we visualized the t(8;14) with a breakpoint at Sα1 in an IgM-expressing BL tumor with a large telomeric 14q deletion affecting the nontranslocated IGH allele. Due to the 14q deletion in this tumor, the VDJ-Cµ/Cδ present on der(8) is the only source of IgM transcription. A breakpoint involving the functional IGH allele was also observed in the DG-75 BL cell line. By use of long-range PCR and DNA sequencing, we showed that the breakpoint was located just upstream of a functional VH3-23/D3-16/JH4-02 rearrangement. By reverse transcriptase-PCR using a VH framework 1 consensus primer and a Cµ primer, we isolated the exact same VDJ sequence, indicating that the IGH allele with the t(8;14) breakpoint was responsible for IgM expression in this cell line (Figure 2, B). It is unclear whether the targeting of the functional IGH allele by chromosomal translocations is a frequent event in BL and, unfortunately, is very difficult to assess. Preferential targeting of the functional IGH allele might imply a predisposition or increased sensitivity, which is probably due to epigenetic factors that influence accessibility and/or localization.

In conclusion, the t(8;14) breakpoint structure involving S regions in human BL differs from S region breakpoint in mouse plasmacytomas, pointing toward a different cell of origin, and does not bear the hallmark characteristics of a canonical CSR-mediated translocation. Furthermore, the nontranslocated IGH allele has not undergone any form of CSR-mediated deletional remodeling. It remains to be established whether CSR is involved in the generation of the t(8;14) in human BL. Due to the reciprocal breakpoint configuration, the t(8;14) in BL can target the functionally rearranged IGH allele without hampering IgM expression.

References


Journal of the National Cancer Institute Monographs, No. 39, 2008 35

Note
Present address: Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA (Jeroen E. J. Guikema).