

Brief report

IgH partner breakpoint sequences provide evidence that AID initiates t(11;14) and t(8;14) chromosomal breaks in mantle cell and Burkitt lymphomas

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Previous studies have implicated activation-induced cytidine deaminase (AID) in B-cell translocations but have failed to identify any association between their chromosomal breakpoints and known AID target sequences. Analysis of 56 unclustered IgH-CCND1 translocations in mantle cell lymphoma across the ~344-kb *bcl-1* breakpoint locus dem-

onstrates that half of the *CCND1* breaks are near CpG dinucleotides. Most of these CpG breaks are at CGC motifs, and half of the remaining breaks are near WGCW, both known AID targets. These findings provide the strongest evidence to date that AID initiates chromosomal breaks in translocations that occur in human bone marrow B-cell progenitors. We also iden-

tify WGCW breaks at the *MYC* locus in Burkitt lymphoma translocations and murine IgH-MYC translocations, both of which arise in mature germinal center B cells. Finally, we propose a developmental model to explain the transition from CpG breaks in early human B-cell progenitors to WGCW breaks in later stage B cells. (*Blood*. 2012;120(14):2864-2867)

Introduction

Most human lymphoma translocations, including the t(14;18)/IgH-BCL2 in follicular lymphoma and the t(11;14)/IgH-CCND1 in mantle cell lymphoma (MCL), appear to arise in B-cell progenitors in the bone marrow and to depend on the RAG complex for chromosome breaks at J_H and D_H segments in the immunoglobulin heavy chain (*IgH*) locus. Almost nothing is known about the chromosomal breakage process at *BCL2*, *CCND1*, or other *IgH* partner loci because no murine or other animal models currently exist for this type of translocation. Recently, we showed that many such breakpoints are specifically targeted to the dinucleotide sequence CpG,^{1,2} and we proposed a chromosome breakage model in which activation-induced cytidine deaminase (AID) creates stable T:G mismatches at methylated CpG sites, followed by RAG cutting of repair intermediates.¹ Here we analyze a large set of “unclustered” t(11;14) breakpoints (ie, those that fall outside of the *CCND1* major translocation cluster [MTC]), and we show that most of these breaks occur at or near known AID hotspots.

Methods

Samples were obtained from the University of Washington Hematopathology Laboratory (22 cases) and the University of Nebraska (10 cases) after approval by the University of Washington Institutional Review Board. t(11;14) breakpoints were mapped using translocation comparative genomic hybridization (translocation CGH).³ Breakpoints were amplified and Sanger sequenced using patient-specific primers (available on request). Statistical methods used to analyze proximity to CpG, and WGCW motifs were described by Tsai et al¹ and in supplemental Methods and supplemental Results (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Results and discussion

We used a modification of array-based CGH called translocation CGH³ to identify and clone 56 unclustered t(11;14) breakpoints in 32 cases of MCL. A total of 55 of the 56 breakpoints (98%) at the *IgH* locus on chromosome 14q32 mapped to a J_H, D_H, or V_H gene segment and showed features consistent with RAG-dependent breakage during V(D)J recombination (see Table 1 and supplemental Tables 1-4, and supplemental Figure 2). The breakpoints on chromosome 11q13 span 329 kb of the 344-kb *bcl-1* breakpoint region between *CCND1* and *MYEOV*, the closest gene centromeric to *CCND1* (Figure 1A). The most centromeric breakpoint was located 12 kb from *MYEOV*, 331 kb from *CCND1*, and > 200 kb from the most distant known breakpoint (Table 1).^{4,5} The most telomeric breakpoint was only 2 kb from the *CCND1* gene. Previous studies⁷⁻⁹ have suggested that the majority of t(11;14) breakpoints are telomeric to the MTC, but 12 (37%) of our 32 cases had breakpoints centromeric to the MTC. Nevertheless, the other 20 cases (63%) had breakpoints telomeric to the MTC, significantly more than would be expected if the breakpoints were equally distributed across the entire *bcl-1* region ($P = .0003$, binomial test).

In 16 of 32 cases (50%), one or both *CCND1* breakpoints were ≤ 4 nt from a CpG dinucleotide (referred to below as CpG breakpoints), including 10 cases (31%) with breakpoints directly at a CpG. The majority of the CpG breakpoints (10 of 16, 63%) were located at CGC (Table 1), a known AID hotspot that is distinctive for not conforming to the consensus WRC motif for AID-dependent DNA deamination.^{10,11} In the remaining 16 cases (50%), the *CCND1* breakpoints were ≥ 10 nucleotides (nt) from the

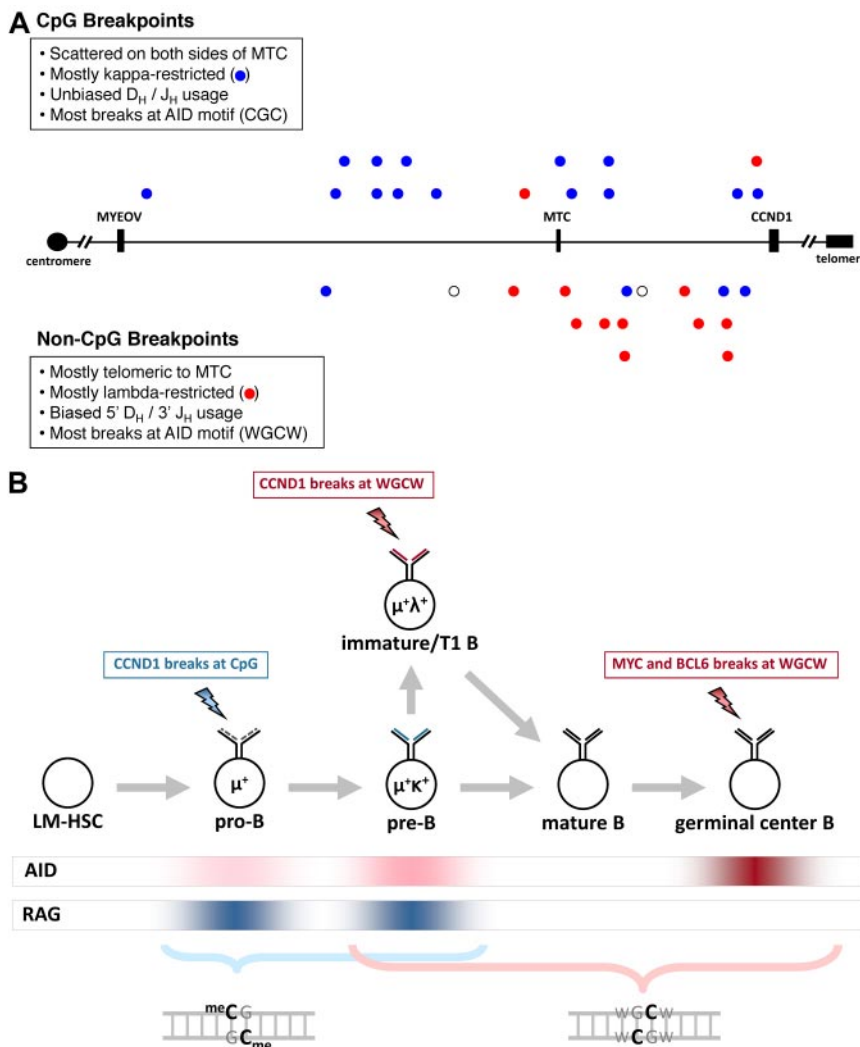
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Figure 1. Distribution and features of unclustered *CCND1* breakpoints and a model for CpG and WGCW breaks in *IgH* translocations. (A) A 400-kb segment of chromosome 11q13 (chr11:68 800 000-69 200 000 in NCBI Build 36) is shown. The *MYEOV* and *CCND1* genes and MTC are shown for reference. CpG breakpoints are shown above the axis, and non-CpG breakpoints are shown below. Blue represents κ -restricted cases; red, λ -restricted cases; and \circ , cases for which light chain restriction is unknown. (B) Our results implicate AID-initiated chromosomal breaks in the genesis of IgH-*CCND1* translocations in MCL, IgH-MYC translocations in Burkitt lymphoma, and IgH-BCL6 translocations in follicular and diffuse large B-cell lymphomas (see supplemental Results). AID expression is highest in antigen-stimulated mature B cells undergoing somatic hypermutation and CSR but is expressed at lower levels in immature and transitional 1 (T1) B cells, and at even lower levels in pro-B and pre-B cells. RAG activity is associated with V(D)J recombination in pro-B cells during *IgH* rearrangement, in pre-B cells during light chain rearrangement, and in immature B cells during receptor editing. Lightning bolts indicate the stage at which each translocation is proposed to occur. Our model proposes that low levels of AID favor CpG breaks (blue font), whereas intermediate or high levels of AID favor WGCW breaks (red). The staggered cytosine bases on opposite strands of the CpG and WGCW motifs are highlighted to indicate the potential of this configuration to promote DSBs.



closest CpG (“non-CpG” breakpoints), including 5 cases for which der(11) breakpoint sequences were unavailable. *CCND1* breakpoints were not associated with any other dinucleotide or RAG motifs (supplemental Table 5A).

The CpG and non-CpG breaks were differentially distributed across the *bcl-1* breakpoint region (Figure 1A). The breakpoints in 13 (81%) of the 16 non-CpG cases were telomeric to the MTC, significantly more than the 5 of 16 (32%) expected if equally distributed across the *bcl-1* region ($P = .00006$, binomial test). In contrast, the CpG breakpoints appear to be randomly distributed across both centromeric and telomeric regions ($P = .2$, binomial). Thus, the overall predominance of telomeric *bcl-1* breakpoints in our series can be explained by the non-CpG breaks alone. This differential distribution of CpG and non-CpG breakpoints suggested that these 2 types of breaks are biologically distinct.

Most B-cell lymphomas show a slight preference for immunoglobulin κ over λ light chain restriction, presumably reflecting the sequential rearrangement of the κ locus before the λ locus during normal B-cell development.^{12,13} To explore the unusual λ restriction bias that has been observed for MCL,¹⁴ we compared light chain restriction status to *CCND1* breakpoint type and location. Ten (71%) of the 14 non-CpG cases for which light chain data were available (Table 1) were λ -restricted compared with only 2 of 16 CpG cases (12%), indicating a strong association between

CpG status and light chain restriction ($P = .0022$, 2-tailed Fisher exact test).

Non-CpG breakpoints also were significantly more likely than CpG breakpoints to involve the most 3' J_H segment (J_H6) and the most 5' D_H segment (D_H2-2; supplemental Tables 2 and 4). J_H6 was rearranged in 8 of 14 non-CpG cases (57%) but in only 2 of 16 (11%) CpG cases ($P = .019$ by Fisher exact test). Similarly, D_H2-2 was rearranged in 4 of 10 non-CpG cases (40%) compared with 0 of 12 CpG cases ($P = .029$). Three of the 4 D_H2-2 cases had J_H6 rearranged on the der(14) chromosome and the remaining case had a D_H2-2 signal joint at the der(14) breakpoint (Table 1). Biased usage of 3' J_H segments and 5' D_H segments has been previously identified at t(14;18) breakpoints¹⁵ but not for t(11;14) breakpoints at the MTC.¹⁶ Taken together, the preferential use of λ light chain and the biased usage of 3' J_H and 5' D_H segments in non-CpG cases suggest that these rearrangements occur at a later stage of B-cell development than CpG breakpoints.

To explore whether AID is involved in generating the non-CpG breaks, we examined the proximity of these breaks to the sequence motif WGCW, where W = A or T. WGCW was recently shown to facilitate AID-dependent double-strand breaks (DSBs) during physiologic class-switch recombination (CSR) by providing 2 antiparallel AID hotspot motifs (WRC, where R = A or G).¹⁷ Strikingly, 12 of the 27 non-CpG breaks at

Table 1. Characteristics of t(11;14) breakpoints

	Case	κ/λ	IgH	der(14) breakpoints					der(11) breakpoints		
				CCND1	Distance to CCND1	Nearest CpG	CGC?	Nearest WGCW	CCND1	IgH	
CpG breakpoints	W7	κ	J _H 5	68 833 760	331 294	1	Y	—	68 833 763	D _H 4-17	
	W8	κ	J _H 6	68 935 608	229 446	0	N	—	68 935 595	D _H 3-3	
	W18	κ	J _H 5	68 940 344	224 710	0	Y	—	68 940 341	D _H 3-10	
	W15	κ	J _H 6	68 957 805*	207 249	0	Y	—	68 957 803	D _H 3-3	
	W19	κ	J _H 4	68 957 805*	207 249	0	Y	—	—	—	
	W17	κ	J _H 4	68 969 141	195 913	0	Y	—	68 969 139	D _H 1–26	
	N1	κ	J _H 4	68 973 670	191 384	0	Y	—	68 973 668	D _H 3-9	
	W13	κ	J _H 5	68 989 831	175 223	2	Y	—	—	—	
	N8	λ	J _H 1	69 037 471†	127 583	0	N	—	69 037 466	D _H 3-22	
	W10	κ	J _H 4	69 056 460	108 594	2	N	—	69 056 458	D _H 2-21	
	N6	κ	J _H 4	69 062 733	102 321	1	N	—	69 062 741	D _H 3-3	
	W11	κ	J _H 4	69 082 644	82 410	0	N	—	—	—	
	W14	κ	J _H 5	69 082 854‡	82 200	2	Y	—	69 082 840	D _H 5-5	
	W16	κ	J _H 4	69 152 043	13 011	4	Y	—	69 152 034	V _H 4-34	
	N2	λ	J _H 4	69 162 371	2 683	0	Y	—	69 162 368	D _H 5-12	
	W22	κ	J _H 1	69 163 029	2 025	0	N	—	69 163 022	D _H 3-10	
	Non-CpG breakpoints	W3	κ	J _H 6	68 930 336	234 718	18	—	3	—	—
		N10	—	J _H 6	68 999 340	165 714	29	—	14	68 999 340	D _H 3-3
N3		λ	J _H 4	69 031 511	133 543	68	—	1	69 031 503	J _H 4s§	
W6		λ	J _H 6	69 059 199	105 855	98	—	4	—	—	
W4		λ	J _H 5	69 065 258	99 796	60	—	16	69 065 237	D _H 2-21	
W2		λ	J _H 4	69 080 506	85 548	25	—	1	69 080 980	D _H 4-23	
W12		λ	J _H 6	69 090 218	74 836	167	—	7	69 090 208	D _H 2-2	
N4		λ	J _H 4	69 091 320	73 734	173	—	10	—	—	
N5		κ	J _H 6	69 092 350	72 704	33	—	3	69 092 347	D _H 3-3	
N9		—	D _H 2-2s§	69 100 683	64 371	10	—	0	69 100 678	D _H 2-2	
W5		λ	J _H 6	69 123 555	41 499	15	—	12	—	—	
W1		λ	J _H 4	69 131 130	33 924	207	—	41	69 131 129	D _H 5-18	
W20		κ	J _H 4/J _H 5	69 144 576	20 478	86	—	28	—	—	
W9		λ	J _H 4	69 146 211	18 843	106	—	3	69 146 211	D _H 4-17	
N7		λ	J _H 6	69 146 814	18 240	29	—	36	69 146 895	D _H 2-2	
W21		κ	J _H 6	69 156 220	8 834	242	—	0	69 156 210	D _H 2-2	

— indicates not applicable.

*CCND1 breaks at same CpG.

†CCND1 break at same CpG as patient HO.⁴‡CCND1 break at same CpG as cell line MO1094.⁶

§V(D)J signal join (see supplemental Figures 1 and 2).

||Non-V(D)J break between J_H4 and J_H5 (see supplemental Figure 1).

CCND1 are within 4 nt of a WGCW motif, making it the second-most frequent break site after CpG ($P = .02$; supplemental Figure 4; supplemental Table 5B).

Similarly, we analyzed a large database of 156 human and 299 murine IgH-MYC breakpoints and found a striking proximity of MYC breaks to WGCW in both human Burkitt lymphomas ($P < .0001$) and murine plasmacytomas ($P < .000001$). Because of the large degree of breakpoint heterogeneity around WGCW at both CCND1 and MYC loci, we included breaks occurring within 4 bp of WGCW (see supplemental Figure 4A-C). Neither CCND1 nor MYC breakpoints were associated with other 4-nt sequence motifs having equivalent degeneracy to WGCW ($P \geq .19$), including GWWC, CWWG, SATS, and STAS (S = G or C), each of which contains 2 G:C and 2 A:T base pairs and a central or peripheral palindromic dinucleotide pair (supplemental Table 5B-D).

The proximity of the CCND1 breaks to the known AID motifs CGC and WGCW provides strong and direct evidence that AID cooperates with RAG to initiate IgH-CCND1 translocations in B-cell progenitors. The palindromic nature of CpG and WGCW facilitates chromosome breakage at CCND1 by providing cytosine bases on both strands of the DNA duplex in almost direct

opposition to one another (Figure 1B), thereby increasing the likelihood of a DSB.¹⁷

To explain why MYC breaks occur at WGCW but not at CpG¹ whereas CCND1 breaks can occur at either CpG or WGCW, we propose the following mechanistic and developmental model for IgH translocations (Figure 1B). CpG breaks are favored at the earliest stages of B-cell development when AID is expressed at very low levels¹⁸⁻²² because deamination of methylated CpG gives rise to T:G mismatches that are repaired 2500-fold less efficiently than U:G mismatches at nonmethylated cytosines.¹ The T:G mismatches preferentially predispose to chromosome breaks because they persist for much longer than U:G mismatches, even though it is likely that both T:G and U:G lesions arise at very low levels.

MYC breaks occur at WGCW because this motif is favored at the very high level of AID found in germinal center B cells, as indicated by the strong preference for DSBs at WGCW motifs in the IgH locus during physiologic CSR.¹⁷ We hypothesize that the non-CpG breaks in MCL might occur in immature/transitional 1 (T1) stage B cells, where AID is expressed at an intermediate level¹⁸⁻²² (Figure 1B), presumably in conjunction with receptor editing.²²⁻²⁴ This would explain their preference for WGCW motifs

as well as their D_H/J_H bias and λ light chain preference, in contrast to the unbiased D_H/J_H usage and κ predominance in CpG cases (Figure 1A).

Given the strong evidence linking AID to DNA lesions within actively transcribed genes,^{25,26} it might seem surprising that AID is targeting CpG and WGCW sites that are so far from the *CCND1* gene (Figure 1A). However, several recent genome-wide studies of AID-induced mutations and chromosomal breaks in mature murine B cells²⁶⁻²⁹ clearly show that AID lesions are not limited to genic regions. For example, Klein et al reported that 10%-20% of their AID-dependent translocation hotspots are intergenic.²⁸ Murine models³⁰ and other experimental systems to study chromosomal translocations in B-cell precursors are needed to better understand the mechanisms underlying these most common *IgH* translocations.

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Authorship

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