Developmental propagation of V(D)J recombination-associated DNA breaks and translocations in mature B cells via dicentric chromosomes

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Mature IgM+ B-cell lymphomas that arise in certain ataxia telangiectasia-mutated (ATM)-deficient compound mutant mice harbor translocations that fuse V(D)J recombination-initiated IgH double-strand breaks (DSBs) on chromosome 12 to sequences downstream of c-myc on chromosome 15, generating dicentric chromosomes and c-myc amplification via a breakage-fusion-bridge mechanism. As V(D)J recombination DSBs occur in developing progenitor B cells in the bone marrow, we sought to elucidate a mechanism by which such DSBs contribute to oncogenic translocations/amplifications in mature B cells. For this purpose, we applied high-throughput genome-wide translocation sequencing to study the fate of introduced c-myc DSBs in splenic IgM+ B cells stimulated for activation-induced cytidine deaminase (AID)-dependent IgH class switch recombination (CSR). We found frequent translocations of c-myc DSBs to AID-initiated DSBs in IgH switch regions in wild-type and ATM-deficient B cells. However, c-myc also translocated frequently to newly generated DSBs within a 35-Mb region downstream of IgH in ATM-deficient, but not wild-type, CSR-activated B cells. Moreover, we found such DSBs and translocations in activated B cells that did not express AID or undergo CSR. Our findings indicate that ATM deficiency leads to formation of chromosome 12 dicentrics via recombination-activating gene-initiated DSBs in progenitor B cells and that these dicentrics can be propagated developmentally into mature B cells where they generate new DSBs downstream of IgH via breakage-fusion-bridge cycles. We propose that dicentrics formed by joining V(D)J recombination-associated IgH DSBs to DSBs downstream of c-myc in ATM-deficient B lineage cells similarly contribute to c-myc amplification and mature B-cell lymphomas.

Significance

Antibody production depends on a cut-and-paste genomic rearrangement termed “V(D)J recombination” that takes place during early B-lymphocyte development. Mistakes in V(D)J recombination can lead to chromosomal translocations that activate oncogenes. Such mistakes usually lead to immature B-cell cancers. However, in the absence of the ATM kinase, mice can develop mature B-cell tumors with translocations resulting from V(D)J recombination-associated breaks. Normally persistent chromosome breaks activate cellular checkpoints that eliminate cells harboring such dangerous lesions. The current studies reveal that, in the absence of ATM, V(D)J recombination-generated breaks are cycled into aberrant chromosomes, termed “dicentrics,” that avoid checkpoints and are propagated through development, generating new breaks and translocations in mature B cells.


The authors declare no conflict of interest.

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Data deposition: The sequence reported in this paper has been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE58599).

References

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Both V(DJ) recombination- and IgH CSR-associated DSBs, which occur in the G1 cell-cycle phase, are joined by the classical nonhomologous DNA end-joining pathway (C-NHEJ), one of the major forms of cellular DSB repair (20, 21). In the absence of C-NHEJ, V(DJ) recombination DSBs are not joined in progenitor (pro)-B cells, and a substantial number of CSR DSBs are not joined in CSR-activated mature B cells, leading to chromosome breaks and translocations (5). However, C-NHEJ-deficient mice are not prone to lymphoma development because proliferation of cells bearing persistent DSBs or oncogenic translocations is prevented by the p53-dependent cell-cycle checkpoint (22, 23). Indeed, C-NHEJ/p53 dual deficiency in mice leads to inevitable pro-B-cell lymphomas carrying translocations that join RAG-initiated DSBs at the IgH J4 locus to DSBs downstream of c-myc, leading to c-myc amplification via a breakage-fusion-bridge (BFB) mechanism (24, 25). Mechanistically, it appears that C-NHEJ deficiency leads to persistent RAG-initiated J4 breaks that, in the absence of p53 are replicated, and thereby can be converted into dicentric chromosomes, which promote the downstream translocation/amplification process (24, 25). Notably, C-NHEJ/p53 double deficiency in murine mature B cells leads to peripheral B-cell lymphomas that harbor IgH/c-myc translocations in which S-region DSBs are fused to sequences upstream of c-myc, leading to c-myc overexpression by juxtaposition to the IgH 3’ regulatory region (26, 27).

In humans, ATM deficiency leads to both T- and B-cell lymphoma. In mice, however, ATM deficiency alone does not lead to B-cell lymphoma but does promote recurrent thymic lymphomas (28, 29). These tumors frequently harbor complex chromosome 14 translocations that are generated by dicentrics that arise during TCRδ locus V(DJ) recombination, often from D6 DSBs, and that lead to amplification of genes upstream of TCRδ (30). ATM-deficient thymic lymphomas also harbor mechanistically related T(12;14) translocations that fuse TCRδ breaks to chromosome 12 sequences over a large region downstream of IgH, leading to deletion of potential tumor suppressors, including Bcl11b (30, 31). Human T-ALLs also frequently harbor translocations initiated from D6 (10). Notably, the translocations in ATM-deficient mouse thymic lymphomas appear to arise via mechanisms reminiscent of those found for C-NHEJ/p53-deficient pro-B-cell lymphomas. This mechanistic overlap likely occurs because ATM deficiency alone produces defects that are caused by combined C-NHEJ and p53 deficiency, including a DSB repair defect, albeit not as severe as that of C-NHEJ deficiency, and a G1 checkpoint defect (16, 17). In this regard, in ATM-deficient mice, RAG-dependent IgH locus breaks that are generated in pro-B cells can persist through development and be manifested in mature B cells as chromosome 12 centromeric fragments that end within IgH or within downstream regions and that lack upstream (telomeric) sequences (17). These chromosome fragments, which can participate in translocations in mature B cells, have been proposed to often lose IgH and variable amounts of downstream sequences due to nucleosome-mediated end erosion in the absence of protective telomeres (17).

Given the potential for both unrepaired V(DJ) recombination-associated and CSR-associated DSBs, along with G1 checkpoint defects in ATM-deficient B cells, an intriguing question is why ATM-deficient mice do not develop peripheral B-cell lymphomas. In this regard, we recently generated ATM-deficient mouse models that routinely develop peripheral mature B-cell lymphomas and found that they routinely harbor translocations mechanistically related to those of C-NHEJ/p53 pro-B-cell lymphomas, in which RAG-initiated IgH DSBs are joined to sequences downstream of c-myc, leading to dicentrics and c-myc amplification via a BFB mechanism (32). To elucidate how RAG-initiated DSBs could lead to dicentrics and c-myc amplification in peripheral B-cell tumors, we used our high-throughput genome-wide translocation sequencing (HTGTS) approach to assay for sequences that translocate to DSBs within the c-myc gene in wild-type (WT) and ATM-deficient B cells. Our findings point to a mechanism in which RAG-initiated DSBs in ATM-deficient pro-B cells are propagated via dicentric chromosomes and through BFB cycles give rise to new DSBs downstream of IgH in mature B cells.

**Results**

**ATM Suppresses Genome-Wide Translocations in Stimulated B Cells.**

To elucidate potential mechanisms by which ATM deficiency promotes peripheral IgM+ B-cell lymphomas with c-myc amplification that results from dicentric translocations involving apparent V(DJ) recombination-associated IgH DSBs (32), we used HTGTS to assess genome-wide translocation patterns from DSBs introduced into the c-myc gene in WT and ATM-deficient mature IgM+ B cells activated in culture by treatment with α-CD40 plus IL4 to induce IgH class switching from IgM to IgG1 and IgE. For this purpose, we used B cells that harbor a cassette with 25 consecutive I-SceI recognition sites (referred to as the 25xI-SceI cassette) within c-myc intron 1 (33). Upon expression of I-SceI during B-cell activation, I-SceI-specific DSBs generated in the 25xI-SceI c-myc cassette can be used as HTGTS “bait” to capture endogenous prey DSBs to which they translocate genome-wide (33). For these studies, we used primers located about 100 bp centromeric to the 25xI-SceI cassette to isolate translocation junctions involving the 5’ broken end (BE) of the 25xI-SceI cassette DSBs (5’ I-SceI-EBs). Junctions of 5’ BEs are considered in the (+) orientation if prey sequences are read from the junction in a centromere-to-telomere direction and the (−) orientation if read in the opposite direction (33). In all HTGTS experiments described, we performed at least three independent repeats for each genotype under each given experimental condition. As all repeats yielded the same major findings, we pooled results from each set of experimental repeats for presentation. For ready visualization of genome-wide translocation patterns on different chromosomes, we present HTGTS junctions as a dot plot in which each chromosome is divided into 2-Mb bins and the number of junctions within each bin is represented by various numbers of color-coded dots (33) (Fig. 1 and Figs. S1A, S2, and S3A).

Consistent with prior results (33), a large fraction (72%) of 5’ I-SceI-cassette HTGTS junctions from CSR-activated WT B cells maps within 20 kb of the I-SceI break-site within c-myc on chromosome 15, with the majority of these representing rejoinering of single I-SceI DSBs following resection (Fig. 1D and Fig. S1A and B). HTGTS libraries from ATM-deficient B cells had a lower fraction of junctions than WT within this immediate break-site region (51%) and, correspondingly, a higher percentage of junctions in the form of translocations along the length of the break-site chromosome (17 vs. 11% in WT) and to other chromosomes (31 vs. 17% in WT) (Fig. S1B). The increased levels of intrachromosomal and interchromosomal translocation of bait DSBs in ATM-deficient B cells is consistent with aberrant resolution and/or prolonged persistence of DSBs promoting their translocation.

Translocations to known AID-dependent hotspots (33, 34) and cryptic I-SceI sites (33) were only modestly increased or, in some cases, decreased in ATM-deficient vs. WT cells (Fig. 2), perhaps due to competition for joining from increased levels of other persistent DSBs within given chromosomes (5, 35). In this context, the major translocation hotspots in both WT and ATM-deficient-activated B cells were the Sx, Sy1, and Sc regions (Fig. 2A and B), which are the primary targets for AID-initiated DSBs in α-CD40/IL4–stimulated B cells. Notably, however, the relative level of the Sx and Sc region translocation hotspots was slightly diminished by ATM deficiency (Fig. 2B), again potentially because of increased levels of other unresolved DSBs that compete for translocation of c-myc bait DSBs in ATM-deficient cells. Strikingly, although in WT B cells translocations dropped to background levels just downstream of IgH S region hotspots, in...
ATM-deficient B cells a substantial number of translocations, far above background levels, were observed over a 35-Mb region downstream (centromeric) of IgH on chromosome 12 (Fig. 1A and Fig. S1A, indicated by red boxes).

De Novo DSBs Over a 35-Mb Region Downstream of IgH Provide c-myc Translocation Targets in Activated Splenic B Cells. To determine whether the high level of translocation junctions found downstream of the IgH locus in ATM-deficient vs. WT-activated B cells resulted from generation of new DSBs vs. potential erosion of developmentally persistent chromosome 12 centromeric fragments resulting from IgH DSBs as proposed previously (17), we assayed the frequency of junctions in the plus and minus orientations. The erosion model of developmental DSB persistence, in its most simple form (Discussion), predicts occurrence of DSBs only in the minus orientation (17) (Fig. 3A) whereas newly generated DSBs provide both ends for joining and thus result in junctions occurring at similar frequency over the region in both plus and minus orientations (33) (Fig. 3B). Strikingly, we found that the dramatically increased junctions across this large region downstream of IgH in activated ATM-deficient B cells vs. WT B cells were evenly distributed in the plus and minus orientations (Fig. 3C, Top and Middle), demonstrating that they involved de novo prey DSBs introduced during CSR activation of the ATM-deficient B cells. Notably, although DSBs were greatly increased across the region, we did not identify any focal hotspots.

There is no obvious mechanism by which off-target activities of AID or RAG could be imagined to generate DSBs over such a large and specific chromosomal region in the absence of ATM. However, we previously found that ATM-deficient B cells activated for CSR generated substantial numbers of chromosome 12 dicentric translocations (18), suggesting that some newly generated DSBs downstream of IgH in ATM-deficient B cells could arise de novo during activation and cell division via breakage of chromosome 12-based dicentrics generated via aberrant CSR. To test whether the downstream IgH DSBs could occur secondary to dicentrics that arise during CSR activation, we prepared HTGTS libraries from WT B cells treated with the KU55933 ATM inhibitor (ATMi) during activation with α-CD40/IL4. This ATMi treatment indeed resulted in increased accumulation of plus and minus HTGTS junctions in the 35-Mb region downstream of IgH, showing that the DSBs that led to them did not arise from earlier developmental events, but rather arose from events that occurred in the course of CSR activation. However, relative levels of these downstream HTGTS junctions (DSBs) were lower than those of similarly activated ATM-deficient B cells, consistent with the possibility that some of the downstream DSBs were either generated or predisposed by events that occurred before CSR activation of ATM-deficient B cells (Fig. 3C, Bottom, and Fig. 3D).

Frequent DSBs Downstream of IgH in Splenic ATM-Deficient B Cells Do Not Require CSR Activation or AID Induction. To determine whether activated ATM-deficient splenic B cells were also predisposed to

Fig. 1. ATM suppresses widespread translocations downstream of IgH in CSR-activated B cells. (A and B) Profiles of unique translocation junctions on selected chromosomes in HTGTS libraries from α-CD40/IL4–stimulated (A) or RP105-stimulated (B) B cells from WT (shown on left of each chromosome diagram) or ATM-deficient (shown on right of each chromosome diagram) mice, as indicated. Genome-wide translocation patterns (for all chromosomes) are shown in Figs. S1 and S2. Plots represent pooled data from at least three independent experiments, all of which gave similar results. Chromosomes are drawn with centromere (Cen.) on top and telomere (Tel.) on the bottom. The blue box on chromosome 15 indicates the break-site. The red box on chromosome 12 indicates the 35-Mb region centromeric to IgH that contains frequent translocation junctions in ATM-deficient activated B cells. Triangles indicate known AID-dependent hotspots (blue) (33 and 34) or cryptic I-SceI sites (green) (33). Numbers of translocation hits within each 2-Mb bin are represented by colored dots, as keyed in the box.
generating AID-independent DSBs downstream of IgH that could contribute to dicentric translocations, we performed HTGTS on WT and ATM-deficient B cells during expansion in the presence of RP105 (Fig. 1B and Fig. S2), which allows B cells to proliferate and survive in culture without expressing AID or undergoing CSR (17). Consistent with lack of AID expression, HTGTS libraries from RP105-treated WT and ATM-deficient B cells had no detectable genome-wide AID hotspots, with the few junctions found in S regions occurring at the very low levels observed in AID-deficient cells (33) (Fig. 2 A and B). However, HTGTS libraries from RP105-treated WT and ATM-deficient cells did retain similar levels of cryptic I-SceI HTGTS hotspots as found in libraries from α-CD40/IL4-activated cells (Fig. 2C), showing that RP105 stimulation did not affect translocations more generally. Notably, although libraries from RP105-stimulated WT B cells had only background levels of HTGTS junctions in the 35-Mb region downstream of IgH, similar to those observed in α-CD40/IL4-activated WT B cells (Fig. 1B, Fig. 4A, and Fig. S2), RP105-stimulated, ATM-deficient B cells retained substantial levels of plus-and-minus-orientation HTGTS junctions over this 35-Mb region (Fig. 4A), with a distribution similar to that of the DSBs that occurred during the course of CSR (compare Fig. 3C and 4A). To further verify increased HTGTS junctions downstream of IgH derived from DSBs generated in ATM-deficient B cells during early development rather than during activation in culture, we generated HTGTS libraries from ATM-treated WT B cells stimulated with RP105. Under these culture conditions, we observed significant levels of cell death and a higher level of HTGTS junctions genome-wide, the latter likely due to increased random DSBs and translocations in dying cells (Fig. S3A). This “background” made it hard to normalise to other libraries; however, it still was quite clear that the 35-Mb region downstream of IgH was not enriched in translocation junctions under these stimulation conditions (Fig. S3B). Taken together, these results indicate that a substantial proportion of DSBs in the IgH downstream region in activated ATM−/− peripheral B cells originate from an AID-independent mechanism.

Discussion

A genetic alteration that increases DSB frequency around c-myc combined with an Eμ-Bcl2 transgene that enhances B-cell survival leads to the development of mature B-cell lymphomas with nearly 100% penetrance in the context of ATM deficiency (32). Remarkably, all of these ATM-deficient mature B-cell lymphomas harbor oncogenic c-myc amplifications generated by a BFB mechanism from dicentric chromosomes formed through fusion of V(D)J recombination-associated IgH DSBs on chromosome 12 to sequences downstream of c-myc on chromosome 15 (32). The finding of recurrent IgH-c-myc translocations/amplifications in mature B-cell lymphomas via an apparently RAG-associated mechanism was unanticipated and led to our current use of HTGTS to identify potential mechanisms. Our HTGTS studies revealed that mature ATM-deficient B cells, when activated to proliferate in culture, frequently generate translocations between c-myc breaks and sequences spread over a 35-Mb region downstream of IgH. Because these translocations are equally distributed in both plus and minus chromosomal orientations on chromosome 12, they must arise from newly generated DSBs across this large region. As this phenomenon also is observed in B cells activated to proliferate in the absence of AID induction and CSR, we suggest that ATM deficiency allows mature B cells to generate chromosome 12 dicentrics that, via BFB cycles, persist to generate new DSBs and c-myc-activating oncogenic translocations/amplifications that can contribute to the development of mature B-cell lymphomas (Fig. 3B and Fig. S4). The clustering of the BFB breaks closer to the initial dicentric break point is notable and could have a number of conceivable explanations. To our knowledge, our system provides the first experimental approach to elucidate the basis of the central DSB feature of the long-known BFB mechanism (36).

Prior cytogenetic studies of ATM-deficient B cells detected large chromosome 12 centromeric fragments, in some cases containing downstream IgH sequences and in other cases lacking IgH sequences, and showed further that presence of such chromosome 12 fragments was AID-independent but RAG2-dependent (17). The model proposed to explain these observations was that, in the
in ATM-deficient cells, which lack the G1 checkpoint, the BFB mechanism that we propose would prevent activation of post-replicative checkpoints and thereby propagate DSBs during cell division that contribute to translocations and gene amplifications, as has been observed in C-NHEJ/p53 doubly deficient pro-B cells (24, 25). In light of our current findings, and given the above considerations, we propose that a dicentric/BFB mechanism likely contributes majorly to developmental propagation of RAG-generated DSBs.

Our findings also may help explain the origin of recurrent chromosomal aberrations in ATM-deficient mouse thymic lymphomas. These tumors frequently harbor complex chromosome 14 translocations involving dicentrics generated during TCRβ V(D)J recombination that amplify genes upstream of TCRβ, as well as T (12;14) translocations that fuse TCRβ breaks on chromosome 14 to chromosome 12 sequences over a large region downstream of IgH, which may result in the deletion of putative tumor suppressors (27). RAG-initiated DSBs in the JH locus occur in developing T cells, and, in the absence of ATM, have been proposed to persist in developing and peripheral T cells as chromosome 12 centromeric fragments generated via exonucleolytic processing of broken ends, similar to what is observed in ATM-deficient B cells (42). Again, however, we note that these findings are consistent with persistence of JH breaks via the dicentric/BFB mechanism, which would provide substrates for translocation to persistent RAG-initiated TCRβ breaks also propagated via BFB cycles. Our findings may also be relevant to the mechanisms of translocations and amplifications observed in mantle cell lymphomas that have somatic ATM inactivation (32). In theory, in permissive backgrounds, DSBs that are propagated via dicentrics through development in other lineages may contribute to cancers beyond those of the immune system.

**Experimental Procedures**

**Mouse Strains Used.** Previously generated c-myc54-5S-I-SceI mice (33) were bred into the ATM-deficient background (43). All mice used were heterozygous for modified alleles containing I-SceI cassettes. All animal experiments were

absence of ATM, unrepaired RAG-generated IgH DSBs in pro-B cells persist as large centromeric chromosome fragments to the mature B-cell stage, but extensive erosion of the broken DNA ends due to lack of telomeres over time leads to loss of sequences centromeric to the IgH locus (17). We note, however, that our currently proposed dicentric model is consistent with all of the earlier cytogenetic findings that led to the erosion model for developmental DSB persistence in the absence of ATM. In addition, although the ATM/p53 pathway is involved in checkpoint activation throughout the cell cycle (37, 38), cells lacking ATM mostly accumulate chromosome breaks, indicating a more prominent role of ATM in detection and repair of prereplicative DSBs (39, 40), with unrepaired DSBs and unprotected chromosome ends in S- and G2/M-activating ATM-independent postreplicative checkpoints (36, 37). In this context, chromosomes containing translocation/amplifications generated via BFB are routinely “stabilized” by acquiring a new telomere (25, 41). Therefore, in ATM-deficient cells, which lack the G1 checkpoint, the BFB mechanism that we propose would prevent activation of post-replicative checkpoints and thereby propagate DSBs during cell division that contribute to translocations and gene amplifications, as has been observed in C-NHEJ/p53 doubly deficient pro-B cells (24, 25). In light of our current findings, and given the above considerations, we propose that a dicentric/BFB mechanism likely contributes majorly to developmental propagation of RAG-generated DSBs.

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performed under protocols approved by the Institutional Animal Care and Use Committee of Boston Children’s Hospital.

**Splenlic B Cell Proliferation, Activation in Culture, and Retrospective Transfection.** Splenic B cells were isolated as previously described (9) and cultured in 15% (vol/vol) FBS-containing RPMI medium supplemented with γ-CD40L (1 μg/ml; eBioscience) plus IL4 (20 ng/ml; R&D Systems) or RPMI plus 2 μg/ml eBioscience. Retroviral infection with I-SceI-GFP-expressing retrovirus (33) was performed after 24 h following γ-CD40L stimulation, and cells were harvested at day 4. For γ-CD40L simulation, infection was performed after 48 h and cells were harvested at day 5. For indicated experiments, ATM inhibitor KU55933 (10 μM; Tocris) was added at time of infection and maintained for the whole duration of the stimulation.

**Generation of HTGTS Libraries.** HTGTS libraries were prepared as previously described (33). Nucleotide sequences of junctions within some libraries were generated by 454 (Life Sciences) sequencing, and others were generated by Mi-seq (illumina) sequencing. For each genotype and condition analyzed, libraries sequenced by different methods showed comparable patterns. At least three independent libraries were generated and analyzed for each experimental condition shown and shown to give very similar results for each genotype and experimental condition. Thus, for presentation, these libraries were pooled. Details of the library pools are as follows: α-CD40L/IL4–stimulated WT B cells—four independent libraries sequenced via 454 (total junctions = 6,897/5,897/8,378/4,673); α-CD40L/IL4–stimulated ATM-deficient B cells—three independent libraries sequenced with 454 (total junctions = 7,725/8,445/6,757); α-CD40L/IL4–stimulated and ATM-treated WT B cells—three independent libraries sequenced with 454 (total junctions = 11,528/17,035/18,264); α-CD40L/IL4–stimulated and ATM-deficient B cells were sequenced with Mi-seq and were used only to compare the genome-wide patterns between the two sequencing methods, which were very similar.

**Data Analysis and Normalization.** Sequence data were aligned and analyzed as described (33). For Mi-seq libraries, we further filtered the reads by removing junctions with a >30-bp gap between bait and prey sequences. RP105 stimulation resulted in loss of AID-dependent hotspots (Fig. 2B), but DSBs (hotspots) at cryptic I-SceI sites (33) were found to occur independently of background and stimulation (Fig. 2C). Thus, for comparison, libraries generated under different stimulation conditions were normalized based on the numbers of junctions at cryptic I-SceI sites in each given library.

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Fig. S1. Ataxia telangiectasia-mutated (ATM) suppresses genome-wide translocations in class switch recombination (CSR)-stimulated B cells. (A) Genome-wide profiles of unique translocation junctions in high-throughput genome-wide translocation sequencing (HTGTS) libraries from α-CD40/IL4–stimulated WT (left side of the chromosome diagram) or ATM-deficient (right side of the chromosome diagram) B cells. Plots shown represent pooled data from three or four independent experiments, all of which gave similar patterns. Genotype, treatment, and total number of hits in pooled libraries are showed at the top. The chromosomes are drawn with centromeres (Cen.) on top and telomeres (Tel.) on the bottom, as indicated. The blue box on chromosome 15 indicates the break-site. The red box on chromosome 12 indicates the 35-Mb region centromeric to IgH that contains frequent translocation junctions in ATM-deficient activated B cells. Triangles indicate activation cytidine deaminase-dependent hotspots (blue) (1 and 2) or cryptic I-SceI sites (green) (1). Numbers of translocation hits within a 2-Mb bin are represented by colored dots as keyed in the box. (B) Percentage of HTGTS junctions in a 20-kb region around the c-myc break-site (“brks < 20 kb”) in chromosome 15 excluding the break-site region (“chr15 brks > 20 kb”) and on other chromosomes (“other chrs”) in libraries from α-CD40/IL4–stimulated WT or ATM<sup>−/−</sup> B cells. Data are shown as mean ± SD of three or four independent repeats; ***P < 0.001 by Student’s two-tailed t test.

Fig. S2. Frequent translocations downstream of IgH in activated ATM-deficient B cells activated under conditions that do not induce CSR. Genome-wide profiles of unique translocation junctions in HTGTS libraries from RP105-stimulated WT (left side of the chromosome diagram) or ATM-deficient (right side of the chromosome diagram) B cells are depicted as outlined in the legend to Fig. S1. Plots shown represent pooled data from three independent experiments, all of which gave similar patterns of junctions genome-wide.
Fig. S3. HTGTS analysis of ATM inhibitor (ATMi)-treated B cells stimulated with RP105. (A) Genome-wide profile of unique translocation junctions in HTGTS libraries from ATMi-treated WT B cells activated by RP105. Plots shown represent pooled data from five independent experiments, all of which gave similar patterns of junctions genome-wide. Genotype, treatment, and total number of hits in pooled libraries are shown at the top. The chromosomes are drawn with centromeres (Cen.) on top and telomeres (Tel.) on the bottom, as indicated. The blue box on chromosome 15 indicates the break-site. The red box on chromosome 12 indicates the 35-Mb region centromeric to *IgH* that contains frequent translocation junctions in ATM-deficient activated B cells as indicated in Figs. S1 and S2. Numbers of translocation hits within a 2-Mb bin are represented by colored dots as keyed in the box. (B) Distribution of translocation junctions on the 45-Mb telomeric region of chromosome 12 in ATMi-treated WT B cells stimulated by RP105. Note that we did not normalize these libraries relative to those shown Figs. 3 and 4 due to increased levels of “background” double-strand breaks (DSBs) associated with cell death under these conditions (see text for more details). Schematic of the chromosome 12 region is shown at the top. Red indicates plus-orientation junctions and blue indicates minus-orientation junctions. The y axis shows the number of hits in each 1.2-Mb bin. Numbers above the telomeric peaks indicate the total HTGTS junctions within *IgH* S region.
Fig. S4. Model for contribution of recombination-activating gene (RAG)-initiated breaks to translocation junctions downstream of the \(IgH\) locus in ATM-deficient mature B cells. The absence of the G1 checkpoint and the lack of proper joining of some RAG-initiated \(IgH\) DSBs in ATM-deficient pro-B cells leads to RAG-initiated DSBs in G1 that persist into S phase where, upon DNA replication, they can generate chromosome 12 dicentrics (1). We note that there is no essential aspect of the model that the dicentrics be formed from two copies of the same chromosome (e.g., ref. 1). The formation of dicentrics avoids DSB responses to other checkpoints and developmentally propagates the initial DSBs into new DSBs downstream of \(IgH\) generated via a breakage-fusion-bridge mechanism. Such a mechanism also could lead to the developmental persistence of chromosome 12 DSBs, translocations, and amplifications. Chromosome 12 (“Chr12”) is in blue, and chromosome 15 (“Chr15”) is in green. Red arrows under “Chr15” indicate the position and orientation of the HTGTS primer.