Mechanisms That Can Promote Peripheral B-cell Lymphoma in ATM-Deficient Mice

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Abstract
The Ataxia Telangiectasia–mutated (ATM) kinase senses DNA double-strand breaks (DSB) and facilitates their repair. In humans, ATM deficiency predisposes to B- and T-cell lymphomas, but in mice it leads only to thymic lymphomas. We tested the hypothesis that increased DSB frequency at a cellular oncogene could promote B-cell lymphoma by generating ATM-deficient mice with a V(D)J recombination target (DJβ cassette) within c-myc intron 1 ("DA" mice). We also generated ATM-deficient mice carrying an Eμ-Bcl-2 transgene (AB mice) to test whether enhanced cellular survival could promote B-cell lymphomas. About 30% of DA or AB mice and nearly 100% of mice harboring the combined genotypes (DAB mice) developed mature B-cell lymphomas. In all genotypes, B-cell tumors harbored oncogenic c-myc amplification generated by breakage–fusion–bridge (BFB) from dicentric chromosomes formed through fusion of IgH V(D)J recombination–associated DSBs on chromosome 12 to sequences downstream of c-myc on chromosome 15. AB tumors demonstrate that B lineage cells harboring spontaneous DSBs leading to IgH c-myc dicentrics are blocked from progressing to B-cell lymphomas by cellular apoptotic responses. DA and DAB tumor translocations were strictly linked to the cassette, but occurred downstream, frequently in a 6-kb region adjacent to c-myc that harbors multiple cryptic V(D)J recombination targets, suggesting that bona fide V(D)J target sequences may activate linked cryptic targets. Our findings indicate that ATM deficiency allows IgH V(D)J recombination DSBs in developing B cells to generate dicentric translocations that, via BFB cycles, lead to c-myc–activating oncogenic translocations and amplifications in mature B cells. Cancer Immunol Res; 2(9); 857–66. ©2014 AACR.

Introduction
The B-cell antigen receptor (BCR) and its secreted antibody form are heterodimers comprising immunoglobulin heavy (IgH) and light (IgL) chains. T-cell antigen receptors (TCR) are similarly composed of either αβ or γδ heterodimers. Exons that encode N-terminal variable regions of Ig or TCR chains are assembled in developing bone marrow B cells and developing thymocytes by V(D)J recombination of germline V, D, and J segments (1). V(D)J recombination is initiated in progenitor (pro) B and T cells by the recombination-activating gene 1 and 2 (RAG) endonuclease, which introduces double-strand breaks (DSB) at borders of a pair of V, D, or J segments and short conserved recombination signal sequences (RSS; ref. 2). RAG-initiated DSBs at V, D, or J segments occur in the G1 cell-cycle phase, in which they are joined by classical nonhomologous DNA end-joining (C-NHEJ; ref. 1). IgH V(D)J exons are assembled at the germline JH region just upstream of the Cμ exons, leading to expression of a μ IgH chain (1). Subsequent assembly of an IgL variable region exon and expression of an IgL protein generates IgM, which is expressed on the surface of the resulting mature B cells as a BCR (3).

The C-terminal constant region of IgH chains can be encoded by different sets of exons (Cγ8) that determine antibody class (e.g., IgM, IgG, and IgA; ref. 4). Surface IgM-expressing B cells migrate from the bone marrow to peripheral lymphoid organs, such as the spleen, in which upon antigen stimulation they can undergo IgH class-switch recombination (CSR) or Ig variable region exon somatic hypermutation (SHM). CSR changes antibody effector functions by replacing Cμ exons with one of several sets of Cγ1 exons that lie 100 to 200 kb downstream (4). CSR uses DSBs initiated by activation-induced cytidine deaminase (AID) within large repetitive switch (S) regions that precede the various sets of Cγ1 exons (4). DSBs in the donor Sγ2 are joined to the DSBs in an acceptor S region by end-joining to complete CSR (1). For SHM, AID introduces lesions into variable region exons that are processed into mutations that contribute to BCR affinity maturation (5, 6).

Chromosomal translocations result from joining of two separate DSBs on the same or different chromosomes (1, 7). Depending on which ends of the DSBs are joined, interchromosomal translocations result in the joining of the centromeric portion of one chromosome to the telomeric portion of another or they can be joined to form dicentric chromosomes.

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and/or acentric chromosome fragments (7). The high frequency of RAG-initiated DSBs generated during V(D)J recombination in developing B and T cells provides translocation substrates (8). Indeed, immature B-cell or T-cell lymphomas in humans and certain mouse models can harbor recurrent translocations that fuse RAG-initiated Ig or TCR locus DSBs to oncogenes and/or join them to other genomic DSBs in a way that deletes tumor suppressors (1, 9). Likewise, in B cells activated for CSR, AID-initiated IgH DSBs can serve as intermediates for chromosomal translocations that contribute to peripheral B-cell lymphomas (1, 10). Cryptic RSS or AID-targeting motifs can also lead to off-target RAG or AID activity, respectively, that contributes to translocations (10–14).

C-NHEJ maintains genomic integrity by rejoining DSBs and, thereby, suppressing chromosome breaks and translocations (1, 15). C-NHEJ is important in G1 when homologous recombination is not available to repair DSBs (15, 16). Despite marked genomic instability, C-NHEJ–deficient mice do not routinely develop cancer, at least in part, due to elimination of cells containing unrepaired DSBs by the G1–S cell-cycle checkpoint (8). In this regard, mice with combined deficiency for C-NHEJ and p53, a tumor suppressor that activates the G1 checkpoint (20), develop cancer, at least in part, due to elimination of cells harboring complex IgH translocations with c-myc oncogene amplification, referred to as ‘‘compilons’’ (17). Such compilons derive from the fusion of RAG-initiated IgH DSBs on chromosome 12 to DSBs downstream of c-myc on chromosome 15, generating dicentrics that lead to oncogenic c-myc amplification via a breakage–fusion–bridge (BFB) mechanism (17, 18). A key aspect of compilon formation is persistence of unrepaired RAG-initiated IgH DSBs due to the absence of C-NHEJ and an impaired G1 checkpoint resulting from the absence of p53. Consequently, these unrepaired DSBs are replicated and can form dicentrics that promote BFB-mediated c-myc amplification (17, 18).

Unrepaired DSBs in G1, including V(D)J recombination and CSR-associated DSBs, activate the Ataxia Telangiectasia–mutated (ATM) kinase, which phosphorylates downstream factors that then form complexes in chromatin surrounding DSBs, preventing premature DSB separation and promoting joining by C-NHEJ (1, 19, 20). In addition, this ATM-dependent DSB response activates p53 to enforce the G1 checkpoint (20). Human ATM deficiency leads to ataxia telangiectasia (AT), a condition that includes immunodeficiency and increased predisposition to T-cell and B-cell cancers (21). ATM inactivation in mice recapitulates some aspects of AT, including predisposition to T-cell and B-cell lymphomas (21). ATM deficiency allows unrepaired RAG-initiated IgH DSBs in developing pro-B cells to persist in mature B cells in which they can be visualized as large centric chromosome 12 fragments that participate in chromosomal translocations (25). Yet, ATM deficiency in mice has not been reported to predispose to B-cell lymphoma (22).

We now have generated compound mutant mouse models that recurrently develop mature B-cell lymphomas in association with ATM deficiency. Characterization of these ATM-deficient B-cell lymphomas and the recurrent chromosomal translocations that they harbor suggests an unanticipated mechanism for their origin.

Materials and Methods

Generation of DA, AB, and DAB mice

To generate the DA cohort, ATM+/− mice (26) were crossed to mice heterozygous or homozygous for the c-mycΔ5 allele (27), and the resulting ATM+/− c-mycΔ5/WT/DAB offspring were intercrossed to obtain experimental and control animals. To generate the AB cohort, mice harboring the Eμ-Bcl-2 transgene (28) were bred into the ATM-deficient background. The DAB cohort was generated by crossing ATM+/− c-mycΔ5/DAB mice to ATM+/− Eμ-Bcl-2 mice. A productive Vh1B1-8 knock-in allele (29) was also crossed into the DA and DAB background. All animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee of Boston Children’s Hospital.

Flow-cytometry analysis

Single-cell suspensions from tumor masses and control organs were stained with three sets of anti-mouse antibodies: αB220-CyChrome (eBiosciences), αCD43-FITC (BD Biosciences), and αIgM-RPE (SouthernBiotech); αB220-CyChrome (eBiosciences), αlglc-FITC (BD Biosciences), and αlglc-PE (BD Biosciences); and αCD3e-PECy5 (eBiosciences), αCD4e-PE (eBiosciences), and αCD8a-FTIC (eBiosciences). Data acquisition was performed on a FACScanCalibur flow cytometer equipped with CellQuest software (Becton Dickinson). Analysis was performed with FlowJo software (TreeStar).

Southern blotting

Southern blotting was performed with 10 to 15 µg of genomic DNA isolated from tumor masses or normal control tissues as described previously (30). The JHr–3 probe is a 1.6-kb HindIII/EcoRI fragment downstream of JHr4. The Jx probe is a 1-kb HindIII/BglII fragment downstream of Jx5. The Cμ probe is an 869-bp XbaI/BamHI fragment spanning Cμ exons 1 and 2. The Ij probe is a 1-kb XbaI/PstI fragment. The MycA probe is a 1.7-kb XbaI fragment upstream of c-myc exon 1. The MycD probe is a 900-bp XhoI/BamHI fragment between c-myc exons 1 and 2. The Myc3 probe is a 2.5-kb XhoI/BamHI fragment comprising the end of c-myc exon 3. A PCR fragment containing MDC1 exon 5 was used as a loading control probe.

c-myc amplification quantitation

Intensities of c-myc bands detected by the Myc3 probe were measured by ImageJ software (version 1.48). Fold
amplification = \( \frac{I_{c,m}}{I_{o,m}} / \frac{I_{o}}{I_{o}} \), where \( I_{c,m} \) and \( I_{o,m} \) are the intensities of the sample band and the control band on the Myc\(^3\) Southern blot; \( I_{o} \) and \( I_{o} \) are the intensities of the sample band and the control band on the loading control Southern blot.

**SHM analysis**

The genomic region comprising the rearranged V(D)J exon and the intron downstream of JH4 was PCR amplified from tumor DNA using degenerate oligonucleotides corresponding to different VH families as forward primers and oligonucleotides downstream of JH4 as reverse primers, as described previously (31).

**Comparative genomic hybridization assay**

Comparative genomic hybridization (CGH) was performed as described previously (24). Genomic DNA from the normal kidney was used as control.

**Northern blotting**

Total RNA from tumor and control samples was extracted with TriPure Isolation Reagents following the manufacturer’s instructions (Roche). Northern blot analysis was preformed according to standard procedures with 10\(\mu\)g of total RNA.

**Isolation of translocation junctions**

Tumor genomic DNA was digested with appropriate enzymes (as evaluated by the Southern blot analysis of c-myc rearrangements) and phenol/chloroform purified. Translocation junctions were cloned following a protocol described previously (32), except that the DNA products after emulsion PCR were further amplified through an additional 30 cycles regular PCR with the same primer. Expected PCR bands were separated on 1% agarose gel, extracted, and Sanger sequenced. Sequencing reads from each PCR products were aligned to mouse reference genome (Mouse July 2007-NCBI Build37/mm9) with the BLAST-like alignment tool (BLAT).

**Cytogenetic analyses**

Metaphase spreads were prepared from lymphoma single-cell suspensions cultured for 3 to 6 hours in the presence of colcemid (KaryoMAX Colcemid Solution; GIBCO) according to standard protocols (33). Hybridization of metaphases with mouse spectral karyotyping (SKY) paints or single-chromosome paints specific for chr12 and chr15 and for two-color fluorescent \textit{in situ} hybridization (FISH) assays was performed as described previously (33).

**Results**

**Mouse models for ATM-deficient peripheral B-cell lymphomas**

To test the hypothesis that DSB frequency around oncogenes such as c-myc may be a rate-limiting factor for B-cell lymphoma development in ATM-deficient mice, we generated ATM-deficient mice with a RAG target sequence (DJ\(\beta\) cassette) inserted in intron 1 of c-myc (c-myc\(^{DJ\beta}\) allele). This cassette is cut efficiently by RAG during B-cell development (27). We intercrossed ATM\(^{-/-}\) mice with c-myc\(^{DJ\beta/DJ\beta}\) or c-myc\(^{DJ\beta/+}\) mice to generate ATM\(^{-/-}\) c-myc\(^{DJ\beta/DJ\beta}\) and ATM\(^{-/-}\) c-myc\(^{DJ\beta/+}\) offspring (collectively referred to as DA mice), while ATM\(^{-/-}\), ATM\(^{+/+}\) c-myc\(^{DJ\beta/DJ\beta}\), and ATM\(^{-/-}\) c-myc\(^{DJ\beta/+}\) mice were kept as controls. Of 17 DA mice analyzed, 5 developed IgM\(^+\) peripheral B-cell lymphomas between 12 and 20 weeks, while the rest developed T-cell lymphomas with similar onset time and characteristics to those of ATM\(^{-/-}\) mice (Fig. 1A; Supplementary Table S1). The c-myc\(^{DJ\beta/DJ\beta}\) and ATM\(^{-/-}\) c-myc\(^{DJ\beta/+}\) controls did not succumb to lymphomas or other neoplasias (Fig. 1A; Supplementary Table S1). B-cell lymphomas in the DA cohort presented in peripheral lymph nodes, mesenteric lymph nodes, spleen, and thymus, as well as in liver or kidney (Supplementary Table S2).

To test whether enhanced survival of ATM-deficient B cells could promote B-cell lymphoma, we generated ATM-deficient mice that harbored an Eμ-Bcl-2 transgene. Eμ-Bcl-2 transgenic mice develop peripheral B-cell hyperplasia due to decreased apoptotic death of peripheral B cells (28, 34). For this experiment, ATM\(^{-/-}\) mice were crossed with Eμ-Bcl-2 transgenic mice and resulting ATM\(^{-/-}\) Eμ-Bcl-2 offspring bred to obtain ATM\(^{-/-}\) Eμ-Bcl-2 (referred to as AB) mice, while ATM\(^{-/-}\) mice lacking Eμ-Bcl-2 and ATM\(^{-/-}\) Eμ-Bcl-2 mice were kept as controls. Within a cohort of 10 AB mice, 2 developed IgM\(^+\) peripheral B-cell lymphomas and 1 developed an IgM\(^+\) peripheral B-cell lymphoma between 12 and 17 weeks of age, each
with a similar presentation as DA tumors. An older AB mouse developed what seemed to be an unrelated, IgM+ B lineage tumor (AB36) in the gastrointestinal tract and spleen, while the remainder of the cohort developed standard ATM−/− T-cell lymphomas (Fig. 1B; Supplementary Tables S3 and S4). ATM−/− Eμ-Bcl-2 littermate controls did not succumb to lymphomas or other neoplasias (Fig. 1B; Supplementary Table S3).

We also generated ATM−/− mice that were either heterozygous or homozygous for the c-mycDJ allele and which also carried the Eμ-Bcl-2 transgene (referred to as “DAB” mice). Of 9 DAB mice analyzed, 7 developed IgM+ B-cell lymphomas and another developed a related IgM+ B-cell lymphoma between 7 and 14 weeks of age, all of which had similar characteristics as the predominant DA and AB B-cell tumors (Supplementary Tables S5 and S6; see below). In these cohorts, the ATM−/− c-mycDJ Eμ-Bcl-2 or ATM−/− c-mycDJ control mice again did not develop lymphomas or other neoplasias (Supplementary Table S5). Five DA mice were generated as a by-product of this cohort, and 1 (DA473) developed an IgM+ B-cell lymphoma (Supplementary Table S5). Finally, 3 DAB mice and 1 DA mouse (DA473) also carried a productive V(D)J exon knocked into one of their JH alleles to drive early B-cell development (29); these 4 still developed IgM+ peripheral B-cell lymphomas that seemed very similar in characteristics to the others (Supplementary Table S5).

Most ATM-deficient B-cell lymphomas arise clonally from IgM+ peripheral B cells

Of the 17 related DA, AB, and DAB B-cell lymphomas, 15 expressed surface IgM and either Igκ (13 tumors) or Igλ (2 tumors), while the other 2 (AB225 and DAB361) did not express surface Ig (Supplementary Tables S2, S4, and S6). Southern blotting assays of, respectively, EcoRI- or HindIII-digested tumor DNA for rearrangement of the IgH JH region...
or the \(I_{kx} \) region revealed distinct, clonal rearrangements of both \(J_{f4} \) and one or more clonal rearrangements of \(J_{k} \) in most tumors, confirming monoclonal origin (Fig. 2 and Supplementary Fig. S1). Lack of two distinct rearranged \(\beta_{1} \) bands in some tumors resulted from aberrant V(D)J joins in the absence of ATM that remove the downstream sequences recognized by the probe (see below). These assays also revealed amplification of the \(J_{f1} \) region and adjacent \(C_{i} \) exons in most DA, AB, and DAB B-cell tumors (Fig. 2 and Supplementary Fig. S1). The 2 tumors that lacked surface IgM still had \(J_{f1} \) rearrangements and amplifications, as well as IgL rearrangements and deletions (Fig. 2; Supplementary Fig. S1 and Supplementary Tables S4 and S6), suggesting loss of IgH expression subsequent to a translocation (35). Sequencing of the region downstream of \(J_{f4} \) from 11 of the ATM-deficient B-cell lymphomas revealed no mutations, consistent with origin from peripheral B cells that had not undergone SHM or CSR (Supplementary Tables S2, S4, and S6).

**ATM-deficient B-cell lymphomas harbor complex chromosome 12 to 15 translocations**

Amplification of the \(J_{f1}/C_{i} \) portion of \(IgH \) in conjunction with coamplification of \(c\-myc \) in the context of chromosome 12;15 complicons was previously observed in pro-B–cell lymphomas from C-NHEJ plus p53-deficient mice (17, 18). Southern blot analysis of EcoRI-digested DNA with a probe downstream of \(c\-myc \) exon 3 (\(Myc3'\)) and/or with other probes internal to or upstream of \(c\-myc \) (probes \(MycD \) and \(MyCA \), respectively) revealed \(c\-myc \) amplification in all tumors with \(J_{f1} \) and/or \(C_{i} \) amplification (Fig. 2 and Supplementary Fig. S1). Notably, some tumors had amplified rearranged \(c\-myc\)–hybridizing EcoRI fragments, which in 6 of them comigrated with \(J_{f4+3}\)–hybridizing EcoRI fragment (Fig. 2, red boxes), suggesting that these translocations closely juxtapose \(c\-myc \) to \(IgH \). Additional Southern blotting studies with \(MyCA \) and \(D \) probes also revealed rearrangements in the upstream portion of \(c\-myc \) in several tumors (Supplementary Fig. S1). Consistent with \(c\-myc \) amplification, Northern blot analyses revealed overexpression of \(c\-myc \) transcripts in tumors that showed amplification (Supplementary Fig. S2A).

To characterize translocations cytogenetically, we performed FISH experiments in which several DA, AB, and DAB tumor metaphases were hybridized with paints specific for chromosome 12 (\(IgH \)) and 15 (\(c\-myc \)), respectively. After image acquisition, paint signals were stripped and metaphases reb Mediterraped with bacterial artificial chromosome (BAC) probes specific for the 3′ region of \(IgH \) or for \(c\-myc \). Translocations juxtaposing these two loci cytogenetically were present in all analyzed tumor metaphases, in the form of complications that retained either the centromeric portion of chromosome 12 or that of 15, with the latter metaphases also routinely harboring a T(12;15; Fig. 3A and B; Supplementary Tables S2, S4, and S6). One or the other of these two types of \(IgH\)/\(c\-myc \) complications were found in C-NHEJ/p53-deficient pro-B–cell lymphomas and models for their origins have been discussed (17, 18).

**Amplification of \(c\-myc \) via a BFB mechanism**

We used CGH to examine amplification around the \(c\-myc \) and \(IgH \) loci in selected tumor DNA samples from each cohort. This analysis showed that amplification at \(IgH \) began downstream of the D gene segments in all samples analyzed along with amplification of \(c\-myc \), which in some tumors ended immediately downstream of \(c\-myc \) (DAB494, DAB496, and DAB601), but in others (e.g., DAB64) extended 100 kb downstream into the \(Pet-I \) locus (Supplementary Fig. S2B). In one tumor (DAB496) amplification on chromosome 15 was focused rather precisely on \(c\-myc \) (Supplementary Fig. S2B). Translocation junctions from 8 tumors (DAB361, DAB494, DAB496, DAB601 and DAB538 and DA473, DA64, and DA360) were cloned by a PCR approach and sequenced. Individual translocation breakpoints for each of the tumors involved a sequence lying within or just downstream of \(J_{f1} \) segments on chromosome 12, consistent with derivation from an attempted V(D)J recombination event, fused in “dicentric” orientation to a sequence on chromosome 15 lying within a region spanning from 99 bp to 140 kb downstream of \(c\-myc \) (Fig. 3C; Supplementary Tables S2, S4, and S6). Consistent with Southern blotting and CGH analyses data, chromosome 15 junctions in 6 of 8 tumors (DAB261, DAB494, DAB496, DAB601, and DAB538 and DA473) involved sequences lying within a 6-kb region just downstream of \(c\-myc \) exon 3, whereas 2 other junctions were further downstream in the \(Pet-I \) locus (Fig. 3C; Supplementary Tables S2 and S6). Southern blotting also revealed tumor DA107 to have an amplified junction just downstream of \(c\-myc \) exon 3, whereas 4 additional \(c\-myc\)-amplified tumors (DA403, AB67, AB143, and DAB277) had coamplified downstream \(Pet-I \) sequences, suggesting dicentric translocation junctions likely occurred at least 100 kb downstream of \(c\-myc \) (Supplementary Fig. S2C; Supplementary Tables S2, S4, and S6).

**Downstream translocation junctions are promoted by the upstream \(D\beta \) cassette**

Southern blot analyses with various digest and probe combinations revealed that, in most DA and DAB tumors, the \(D\beta \) cassette had undergone normal or aberrant rearrangements consistent with expected V(D)J recombination–associated events (27) in the absence of ATM (Fig. 4A and B, and Supplementary Tables S2, S4, and S6). However, as the major dicentric translocation junctions that result in \(c\-myc \) amplification in DA and DAB tumors lie downstream of \(c\-myc \), the \(D\beta \) cassette within \(c\-myc \) first intron was not directly involved. To determine whether the \(D\beta \) cassette indirectly influenced translocations linked in cis, we performed Southern blotting with the \(Myc3' \) probe on Kpn1-digested DNA from the nine DA or DAB tumors that were heterozygous for the \(D\beta \) allele. This blotting strategy distinguishes wild-type (WT) from \(c\-myc^{(\beta)} \) alleles. Strikingly, all 9 of these \(c\-myc^{(\beta)}\)/WT tumors had undergone the downstream dicentric translocation on the chromosome 15 containing the \(c\-myc^{(\beta)} \) allele (Fig. 4C). In this regard, it is notable that a substantial fraction of the DAB and DA translocation breakpoints clustered within the 6-kb region just downstream of \(c\-myc \) in the vicinity of a series of
strong cryptic RSSs (Fig. 4D; Supplementary Table S7). Notably, no strong cryptic RSSs were found within the 6.4-kb region containing the *c-myc* gene that lies just upstream (Fig. 4D; ref. 36).

**Discussion**

We have developed the DA, AB, and DAB compound mutant mouse models, which, in the context of ATM deficiency, all develop spontaneous IgM⁺ peripheral B-cell lymphomas that harbor clonal translocations from the *IgH* *JH* region to sequences downstream of *c-myc* to form dicentric derivatives and *IgH/c-myc* amplifications in the context of complicons. The general structure of the *IgH/c-myc* complicons in these tumors is strikingly similar to that of RAG-dependent complicons found in pro-B lymphomas from C-NHEJ/p53-deficient mice (17, 18), with the clustering of *IgH* translocation breakpoints in or near the *JH* region, strongly indicating that the *IgH* complicon breakpoint in ATM-deficient tumors is also RAG initiated. In the absence of C-NHEJ and p53, pro-B cells replicate persistent RAG-initiated *JH* DSBs, cycling them into dicentrics and complicons (17, 18). ATM deficiency results in both V(D)J recombination defects and impaired G₁ checkpoints (20, 25, 37), but, unlike C-NHEJ deficiency, does not block V(D)J joining (37). Thus, we propose that some ATM-deficient pro-B cells generate dicentric chromosomes from replication of persistent RAG-initiated DSBs on one *IgH* allele, but that productive V(D)J rearrangements on the other allele allow such B cells to develop to the IgM⁺ B-cell stage, in which they contribute to B-cell lymphomagenesis. Our finding that incorporating a preassembled *IgH* variable region into the DAB model does not alter the tumor outcome supports this
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Figure 4. Chromosome 15 translocations in DAB and DA tumors involve the c-myc\textsuperscript{DJ\textbeta} allele. A, map of c-myc\textsuperscript{WT} and c-myc\textsuperscript{DJ\textbeta} alleles showing SacI (SI) and KpnI (KI) restriction sites and location of probes. B and C, Southern blot analysis of SacI- (B) or KpnI-digested (C) genomic DNA from DA and DAB tumors with indicated probes. Molecular weight markers and running position of WT c-myc (c-myc\textsuperscript{WT}), nonrearranged c-myc\textsuperscript{DJ\textbeta} (c-myc\textsuperscript{DJ\textbeta}NR) and rearranged c-myc\textsuperscript{DJ\textbeta} (c-myc\textsuperscript{DJ\textbeta}R) alleles are indicated. DNA from 129/Sve spleen (WT SPL) and c-myc\textsuperscript{DJ\textbeta} spleen (DJb SPL) and thymus (DJb THY) was used as controls. B, in the MycD blot, rearrangements of the c-myc\textsuperscript{DJ\textbeta} allele are compatible with precise (496) or aberrant (other tumors) V(D)J recombination joins within the DJ\textbeta cassette in the absence of ATM. C, in the Myc3' blot, tumor 538 shows an amplified and rearranged KpnI fragment derived from the c-myc\textsuperscript{DJ\textbeta} allele, as expected from the cloned translocation breakpoint (Fig. 3C and Supplementary Table S6). Tumors 538 and 806 show amplification with the Myc3' probe but not the MycD probe, consistent with data shown in Fig. 2 and Supplementary Fig. S1. D, schematic showing the positions of tumor translocation junctions (top) and cryptic RSSs (bottom) in the region from c-myc exon 1 to 6 kb downstream and two other short regions more than 100 kb downstream of c-myc. Prediction of cryptic RSSs within this 6 kb downstream region was generated with the program described at http://www.itb.cnr.it/rss/index.html. Note that no strong cryptic RSS was predicted to lie in the 6.4-kb region, spanning the c-myc gene, upstream of the first left cryptic RSS. Blue boxes, c-myc exons; yellow box, the DJ\textbeta cassette. Red triangles, junctions from 1: DAB361, 2: DAB494, 3: DAB496, 4: DAB601, 5: DAB538, 6: DA473, 7: DAB64, and 8: DA360 tumors. Cryptic 23 RSSs are shown in green, and 12 RSSs are shown in black; arrowheads, RSSs in (−) orientation if pointing to left, RSSs in (+) orientation if pointing to the right.
interpretation. Overall, our findings lead us to propose that ATM suppresses mature B-cell lymphomas in susceptible backgrounds by preventing the conversion of unrepaired RAG-initiated IgH breaks in pro-B cells into dicentric chromosomes that, through BFB cycles, give rise to new DNA breaks, translocations, and gene amplifications that contribute to mature B-cell tumors (Fig. 5). We now have provided direct support for this model in parallel studies that used our high-throughput genome-wide translocation sequencing method to study translocations of c-myc DSBs in WT and ATM-deficient B cells (38).

The AB B-cell lymphoma model confirmed that the Eμ-Bcl-2 transgene promotes survival of ATM-deficient developing or mature B cells with the capacity to contribute to mature B-cell lymphomas. On the other hand, the DJβ cassette in the DA and DAB models did not lead to B-cell lymphoma by increasing DSBs and translocations within c-myc intron 1. In human B-cell lymphomas, c-myc intron 1 is a major translocation hotspot (39). However, peripheral B-cell lymphomas that arise in a different mouse model routinely harbor standard IgH/c-myc translocation junctions upstream of the c-myc promoter (30, 40). Together, these findings are consistent with the possibility that translocations into mouse c-myc intron 1 may incapacitate the gene by disrupting the alternative promoter upstream of c-myc exon 2 (41, 42). Yet, the strict linkage of the downstream dicentric translocations in DAB tumors to the c-myc(qβ) allele confirms that the cassette contributes to their appearance. Moreover, the clustering of a substantial fraction of the DAB and DA dicentric junctions within the 6-kb region just downstream of c-myc near strong cryptic RSSs suggests a potential mechanism (Fig. 4D). We propose that the DJβ cassette may contribute to downstream DSBs by providing a bona fide set of proximal 12 of 23 RSSs for pairing with closely linked downstream cryptic RSSs, thereby enhancing RAG cutting at these sites and potentially others within so-called megabase “proximity” domains (1, 43). In the absence of ATM, such RAG-initiated DSBs might be aberrantly repaired (44, 45), with downstream breaks and translocations arising in cells in which cassette DJβ DSBs are resolved within the cassette, thereby preserving c-myc integrity. Notably, dicentric translocations junctions in pro-B lymphomas from C-NHEJ/p53–deficient mice, which do not harbor the DJβ cassette, did not occur within this 6-kb c-myc proximal region, but rather occurred much further downstream (17, 18). Although this finding is consistent with the enhanced RSS cleavage model we propose, we note that...
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...and differences in the effects of C-NHEJ deficiency versus ATM deficiency with respect to repair of particular types of DSBs could also theoretically contribute.

Why do AB, DA, and DAB tumors all arise in association with IgH/c-myc translocations/amplifications generated by the same basic BFB mechanism? The occurrence of AB tumors indicates that B lineage cells harboring spontaneous DSBs that lead to IgH/c-myc dicentrics normally arise in the ATM-deficient background at low frequency, but that their progression into B-cell lymphomas is dampened by cellular responses that lead to apoptotic cell death. On the other hand, introduction of the DJB cassette into intron 1 of c-myc in the ATM-deficient background seems to greatly increase DSB frequency, perhaps at cryptic RSSs downstream of c-myc, thereby potentially increasing dicentric translocations and providing sufficient numbers of cells with dicentric intermediates that some escape cell death. In this scenario, the compound DAB mutant would both increase translocation frequency and allow cells with such translocations to survive, further increasing tumor penetrance to nearly 100%, as observed.

The peripheral DA, AB, and DAB B-cell lymphomas arise from peripheral IgM+ B cells that had not undergone either CSR or SHM. The lack of B-cell lymphomas derived from germinal center (GC) B cells in ATM-deficient mice is surprising, given that activated ATM−/− B cells in culture form AID-initiated dicentrics and other translocations at a high frequency (33). Thus, the lack of such tumors in ATM-deficient mice might result from more strict checkpoints in GC B-cell lymphocytes, which eliminate cells carrying off-target AID DNA damage. In this context, perhaps the susceptible ATM-deficient newly generated IgM+ B-cell population is more tolerant of DNA damage or oncogenic stress than ATM-deficient GC B cells. In addition, progenitors for ATMT−/− IgM+ B-cell lymphomas may arrive in the periphery with activated c-myc genes or c-myc genes predisposed to amplification that allows them to more readily achieve full oncogenic transformation. These findings could be relevant to certain pre-GC human B-cell lymphomas, for example, a subset of mantle cell lymphomas (MCL) derived from pre-GC B cells with ATM mutations (46). MCLs also frequently harbor translocations between chromosomes 11 and 14 that lead to deregulation of the cyclinD1 gene through translocation to the IgH JH region, and a number of MCLs have complex chromosomal rearrangements with coamplified IgH/cyclinD1 genes (47, 48). The potential relevance of our findings for mechanisms of B-cell lymphomagenesis in patients with AT awaits a more extensive and in-depth characterization of these human ATM-deficient B-cell tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Tepsuporn, J. Hu, M. Gostissa, F.W. Alt

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References


12. Greisman HA, Lu Z, Tsai AG, Greiner TC, Yi HS, Lieber MR. IgH partner breakpoint sequences provide evidence that AID initiates t(11;14) and t(8;14) chromosomal breaks in mantle cell and Burkitt lymphomas. Blood 2012;120:2864–7.


28. Tepsuporn et al. –

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Mechanisms That Can Promote Peripheral B-cell Lymphoma in ATM-Deficient Mice

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