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Report

Chimeric IgH-TCR/ translocations in T lymphocytes mediated by RAG

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Translocations involving the T cell receptor alpha/delta (TCR α / δ) chain locus, which bring oncogenes in the proximity of the TCR α enhancer, are one of the hallmark features of human T cell malignancies from ataxia telangiectasia (AT) and non-AT patients. These lesions are frequently generated by the fusion of DNA breaks at the TCR α / δ locus to a disperse region centromeric of the immunoglobulin heavy chain (IgH) locus. Aberrant VDJ joining accounts for TCR α / δ associated DNA cleavage, but the molecular mechanism that leads to generation of the “oncogene partner” DNA break is unclear. Here we show that in ATM deficient primary mouse T cells, IgH/TCR α / δ fusions arise at a remarkably similar frequency as in human AT lymphocytes. Recombinase-activating gene (RAG) is responsible for both TCR α / δ as well as IgH associated breaks on chromosome 12 (Chr12), which are subject to varying degrees of chromosomal degradation. We suggest a new model for how oncogenic translocations can arise from two non-concerted physiological DSBs.

Introduction

One of the most frequent cytogenetic abnormalities found in prolymphocytic leukemia, adult T cell leukemia and in leukemias from AT patients are translocations and inversions between the TCR α locus (14q11) and a disperse region near band 14q32.¹ The breakpoints at 14q32 span a region of 450 kb that is centromeric of the telomere-proximal IgH locus and frequently includes the TCL1 oncogene.^{1,2} In primary lymphocytes from normal individuals, non-clonal t(14;14)(q11;q32) translocations appear at a

frequency of 0.002%.³ AT patients exhibit a 250-fold increase in the probability of developing leukemia or lymphoma, and the frequency of non-clonal t(14;14)(q11;q32) translocations is also increased 250-fold (0.5%) in primary AT lymphocytes.⁴

Atm^{-/-} mice provide a model for studying lymphoid tumor development in AT patients. *Atm*^{-/-} mice are predisposed to thymic lymphomas,⁵ all of which harbor chromosome 14 (Chr14) rearrangements involving the TCR α locus.⁶ TCR α / δ translocations are also common in primary *Atm*^{-/-} T cells,⁶ and these rearrangements are dependent on expression of the RAG endonuclease which introduces DSBs at Ig and TCR loci during V(D)J recombination.⁷ Mouse Chr12 is homologous to human chromosome 14, and is a translocation partner for TCR α / δ in approximately 50% of *Atm*^{-/-} thymic lymphomas.⁶ Analogous to human T cell tumors, the breakpoints on chromosome 12 in *Atm*^{-/-} mouse lymphomas map to a disperse region centromeric of the IgH locus, and there is a frequent loss of the IgH locus on the translocated chromosome.⁶ Since there is a wide dispersion of breakpoints in a region outside of the IgH locus for both human t(14;14)(q11;q32) and mouse T(12;14) translocations, this suggests that unlike the TCR α / δ locus, the “oncogenic” partner might not be targeted by RAG.

Recently, it was shown that ATM functions in two complementary ways to maintain chromosomal integrity during V(D)J recombination.⁷⁻¹¹ In the absence of ATM, the ability to repair antigen receptor coding intermediates at Ig and TCR loci is significantly compromised.^{9,11} In addition to its role in DNA double strand break (DSB) repair, ATM prevents the transmission of DNA double strand breaks (DSBs) to daughter cells,⁷ potentially by activation of a p53 dependent apoptotic checkpoint.¹² Impairment in both of these repair and checkpoint functions likely accounts for the high level of antigen receptor-associated DSBs in mature lymphocytes, some of which are generated earlier in development as a result of failed V(D)J recombination.^{7,12} Although broken antigen receptor loci with telomere-free ends persist through several rounds of cell division, they are characterized by varying degrees of chromosomal erosion.⁷ Prior to loss of essential genetic

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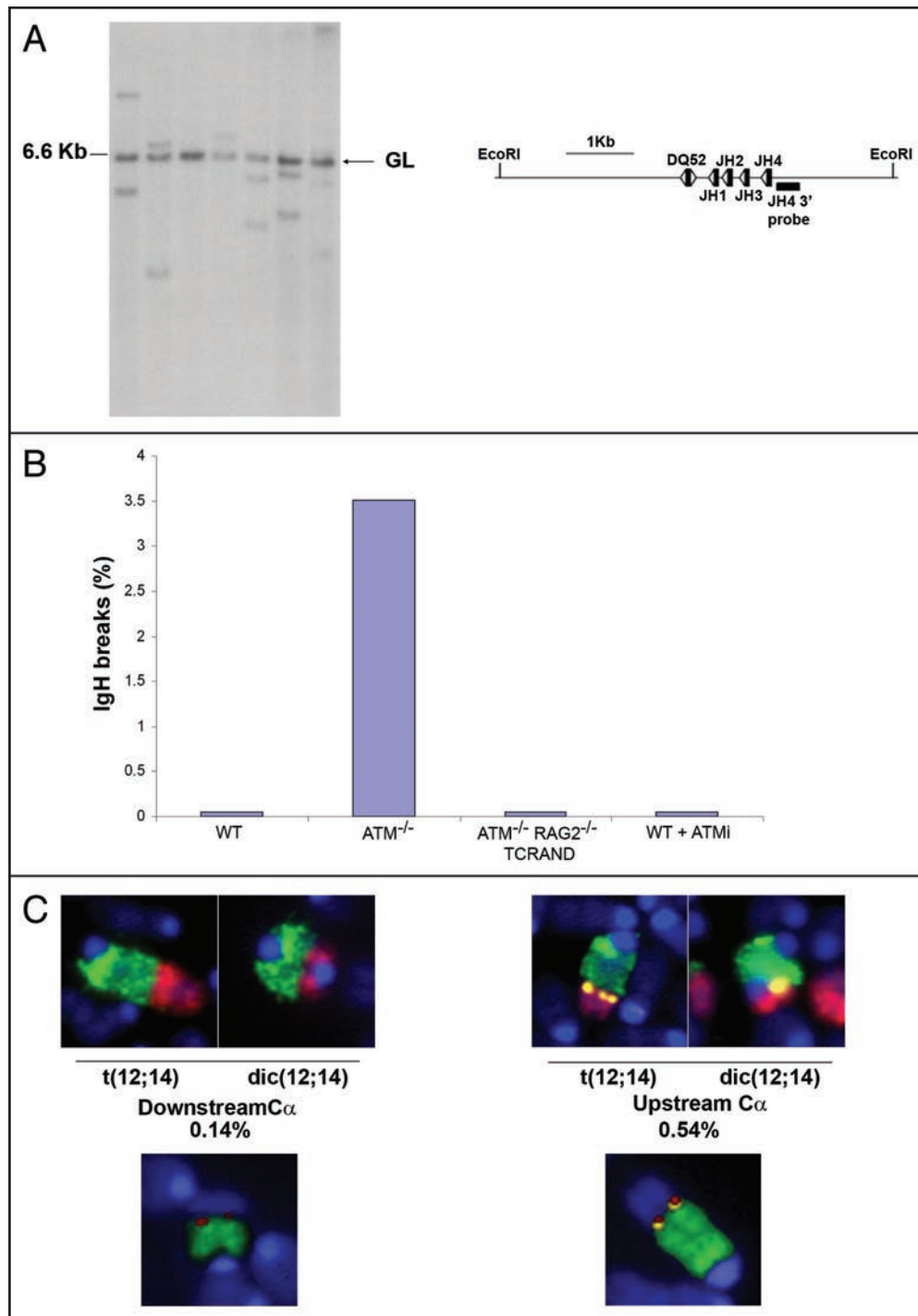


Figure 1. For figure legend, see page 2410.

material, terminally deleted chromosomes have the potential to fuse with chromosomes broken in subsequent generations, as suggested by the increase in IgH associated translocations after irradiation.⁷ Here we show that RAG endonuclease dependent IgH breaks in ATM^{-/-} T cells can persist with varying degree of chromosomal loss and fuse at a high frequency to RAG-dependent TCR α / δ breaks. RAG dependent DSBs on both chromosomes

might also be the source of the highly selected translocation in polyclonal leukemia and adult T cell leukemias.

Results and Discussion

Recombination between immunoglobulin Dh and Jh segments occurs at a high frequency not only in developing B cells but also in early T lineage progenitors in the thymus.¹³⁻¹⁷ Nevertheless,

Figure 1. IgH rearrangements, breaks and translocations in ATM deficient T cells. (A) Southern blot analysis of Ig Dh-Jh rearrangements in mature $\alpha\beta$ T cell hybridomas. Rearrangements were identified by hybridizing EcoRI digested genomic DNA with a probe downstream of Jh4, as indicated on the schematic. Germline band at 6.6 kb is indicated. (B) Percentage of metaphases with abnormalities specifically associated with the IgH locus. Metaphases from *Atm*^{+/+} (WT), *Atm*^{-/-}, *Atm*^{-/-}RAG2^{-/-}TCRAND and WT T cells treated with the ATM inhibitor (ATMi) were prepared 48 hours after lymph node T cell stimulation and analyzed using the IgH/telomere FISH assay as described.¹⁸ At least two independent mice were analyzed for each genotype. (C) Examples of translocations between Chr12 and Chr14. Metaphases were hybridized with a Chr12 painting probe (green), a Chr14 painting probe (red) and a IgH C α BAC probe (yellow), and counterstained with DAPI (blue) (top) or a Chr12 painting probe (green), a TCR α probe (red) and a IgH C α BAC probe (yellow) (lower). Among 2012 metaphase spreads analyzed by FISH, 0.14% showed Chr12/Chr14 fusions in which the breakpoints on Chr12 were centromeric of C α (left), and in 0.54% of cases, C α was precisely at the breakpoint (right). M-FISH analysis estimated Chr12/Chr14 fusions at a somewhat higher frequency (1.11%, see Table 1). FISH-labeled metaphases were imaged using a Zeiss Axiomager M1 wide-field upright epifluorescence microscope (Carl Zeiss Microimaging Inc., Thornwood, NY), equipped with a motorized scanning stage and monochrome progressive scan video CCD camera (JAI America Inc., Woburn, MA), controlled by Metafer image acquisition and metaphase pattern recognition software (Metasystems Group Inc., Watertown, MA). Automated identification of metaphases used the pattern recognition algorithm in the MSearch module of the Metafer software (version 4.0). For M-FISH, metaphases were hybridized with the 21x mouse 21-color probe cocktail from Metasystems GmbH (Watertown, MA), and analyzed using Isis software.

Table 1 Frequencies of translocations involving chromosomes 12 and 14 in ATM^{-/-} T cells (n = 450 metaphases scored, from three experiments)

Type	Translocation	% Frequency
T(14;12) or T(12;14)		1.11%
T(12;x) or T(x;12)	Dic(6;12)	2.22%
	T(12;13)	
	T(12;13)	
	T(12;15)	
	T(10;12)	
	T(19;12)	
	T(12;13)	
	T(10;12)	
	T(10;12)	
	T(10;12)	
T(14;13) or T(13;14)		3.56%
T(14;x) or T(x;14)	T(11;14)	1.78%
	T(14;6)	
	T(8;14)	
	T(14;3)	
	T(14;9)	
	Dic(14;14)	
	T(6;14)	
	Dic(14;14)	

T(12;x) indicates partners for chromosome 12 translocations, excluding chromosome 14. T(14;x) indicates partners for chromosome 14 translocations, excluding chromosome 12.

productive assembly of Ig genes is restricted to B cells, since Ig variable Vh segments are not recombined in T cells. To determine the frequency of Dh-Jh rearrangements in ATM deficient cells, we performed Southern blot analysis on mature T cell hybridomas generated from mature $\alpha\beta$ T cells from two *Atm*^{-/-} mice. All of the 257 hybridomas analyzed gave a 6.6 kb band indicative of an un-rearranged IgH allele, as would be expected given that both IgH alleles of the BW5147 fusion partner are un-rearranged. However, 126 of the hybridoma clones had one or two hybridizing fragments

of diverse sizes indicative of Dh-Jh rearrangements (Fig. 1A). Among these 126 clones, 59 had two diverse fragments indicating that in these cells both IgH alleles in the $\alpha\beta$ T cells had undergone rearrangements. Thus, at least 49% of ATM deficient T cells have undergone Dh-Jh rearrangements on one or both alleles.

Since failed V(D)J recombination intermediates are transmitted to downstream progenitors in the absence of ATM,⁷ we speculated that aberrant Ig Dh-Jh rearrangements generated in immature *Atm*^{-/-} T cells might accumulate as IgH-associated breaks in mature T cells. To test this, we analyzed metaphase spreads from day two *Atm*^{-/-} lymph node T cells by fluorescence in situ hybridization (FISH) with probes specific for chromosome 12 (Chr12), IgH C α , and telomere repeats.^{18,19} Amongst 199 metaphases analyzed from two independent *Atm*^{-/-} mice, seven of them (3.5%) showed IgH associated chromosome breaks evidenced by loss of telomeric sequences (Fig. 1B). In two of the aberrant metaphases, there was also a failure to hybridize with the IgH C α probe, which is centromeric of the VDJ cluster. Cytogenetic analysis using DAPI-banding revealed that both of these breaks localized near band F1, which is the same Chr12 region observed in some of the t(12;14) translocations in *Atm*^{-/-} tumors.⁶ In contrast to *Atm*^{-/-} cells, the IgH locus was intact in all of the T cells derived from WT littermate controls (n = 200 metaphases) (Fig. 1B).

To determine whether Ig heavy chain specific chromosome breaks in mature T cells are products of V(D)J recombination, we performed the IgH FISH assay on metaphase spreads from *Atm*^{-/-} Rag2^{-/-}TCRAND T cells.⁷ TCRAND is a transgenic T cell receptor that reconstitutes T cell development and generates mature clonotypic T cells in the absence of RAG.²⁰ In contrast to lymph node T cells from *Atm*^{-/-} mice, no IgH chromosome breaks were found in T cells from *Atm*^{-/-} Rag2^{-/-}TCRAND mice (n = 70 metaphases) (Fig. 1B). Thus, IgH-associated DNA damage in mature *Atm*^{-/-} T cells is dependent on RAG mediated cleavage. To control for the possibility that IgH breaks in ATM deficient cells are generated by RAG re-expression in mature T cells, we treated WT lymph node T cells with KU55933 (ATMi), a small molecule inhibitor of ATM kinase activity. This treatment increased the total number of aberrant metaphases to levels similar to those found in *Atm*^{-/-} T cells (data not shown). However, unlike WT B cells stimulated to undergo class switch recombination,⁷ treatment of T cells with ATMi did not produce IgH-specific DNA damage (n = 106 meta-

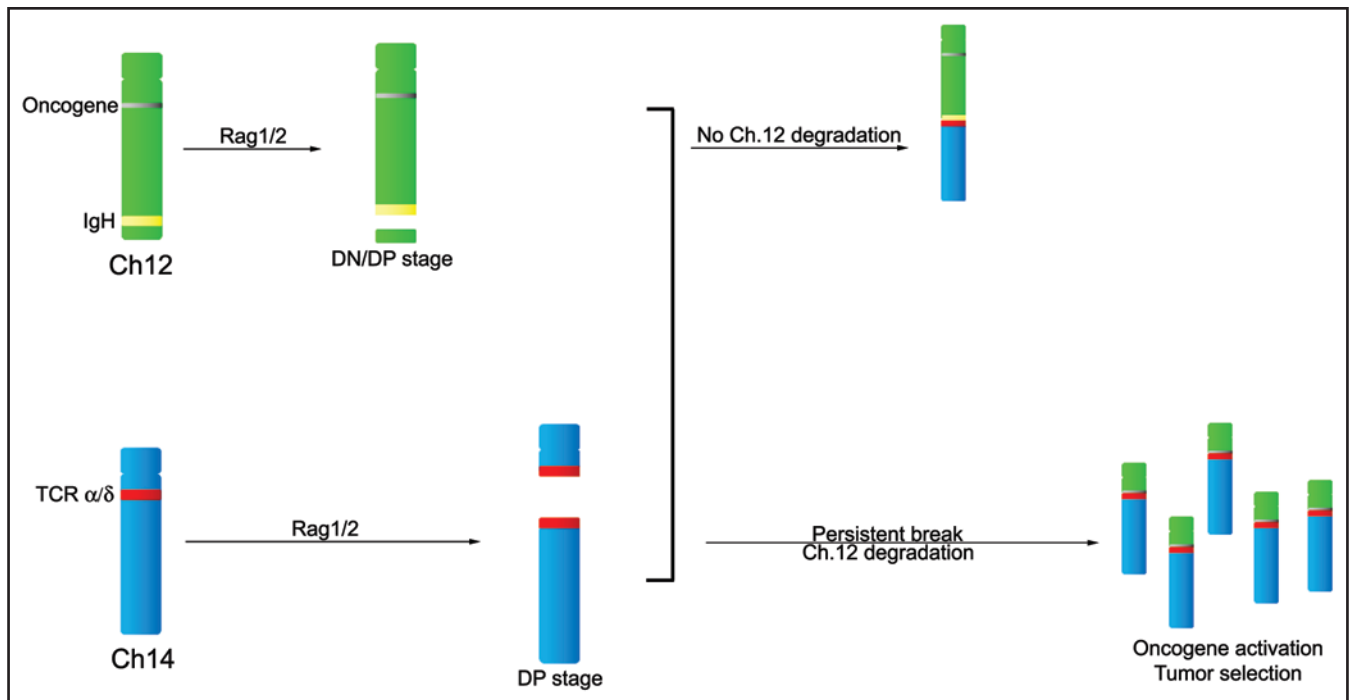


Figure 2. Model for translocations in ATM deficient T cells. Immunoglobulin Ig Dh-Jh recombination associated DSB in immature (DN/DP CD4^{-/-}CD8^{-/-}/CD4⁺CD8⁺) thymocytes may persist until they translocate to the TCR α/δ locus during failed V α -J α or V δ -D δ J δ recombination. Oncogenes localized centromeric of IgH might be brought into the vicinity of the TCR α enhancer (or other element) if the unprotected telomere-free Chr12 end is subject to degradation.

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phases) (Fig. 1B). Thus, IgH associated breaks, characterized by varying degrees of chromosomal loss, are not caused by de novo cleavage in mature ATM deficient cells, but more likely, they are produced earlier as a result of failed Dh-Jh recombination during thymocyte development. Nevertheless, these RAG-dependent chromosome breaks initiated in the thymus persist in the peripheral T cell compartment.

RAG-dependent IgH breaks in ATM deficient T cells occur at a frequency of 3.5% (Fig. 1B), while RAG-dependent TCR α/δ breaks are found in 5.8% of metaphases ($n = 1212$ metaphases analyzed, not shown). Given the high level of unresolved/persistent breaks, we wished to determine the frequency of translocation between these loci. Metaphase spreads from *Atm*^{-/-} T cells lymph node cells were identified using an automated image acquisition system and image analysis software (for details see Materials and Methods). Among 2012 metaphase spreads analyzed by FISH with probes specific for Chr12, Chr14 and IgH C α , 14 of them (0.68%) harbored t(12;14) translocations or dic(12;14) dicentric chromosomes (Fig. 1C). This is remarkably similar to the incidence of t(14;14)(q11;q32) translocations (0.5%) or inv(14) inversions (0.65%) found in human AT lymphocytes.⁴ In three of 14 translocations, the breakpoints on Chr12 were located centromeric of C α (Fig. 1C), consistent with chromosomal degradation. Using probes specific for TCR α , Chr12 and IgH C α , we detected fusions in which TCR α /IgHC α were at the breakpoint and fusions between TCR α and Chr12, in which C α hybridization was lost (Fig. 1C).

The frequency of the Chr14-Chr12 translocation in primary ATM^{-/-} T cells produced by RAG mediated cleavage of both chromosomes (6.9×10^{-3}) is 2–3 orders of magnitude higher than c-myc/IgH translocations induced in primary *Atm*^{-/-} B cells (2×10^{-6}) by AID dependent DSBs,¹⁸ or by I-SCE1 or RAG endonuclease translocation reporters^{21,22} (3×10^{-5}). To determine whether Chr12 and Chr14 were indeed favored partners for translocation, we used multi-color FISH (M-FISH) to karyotype metaphases from 3 independent ATM^{-/-} mice (Table 1). Among 450 metaphases examined, 61 (13.5%) exhibited translocations. Among these translocations, 25 (41%) involved Chr14 and 19 (31%) involved Chr12; therefore these two chromosomes are involved in 78.5% of all translocations. Fusions between Chr12 and Chr14 were highly favored, as 17% of all Chr14 translocations involved Chr12, and 26% of all Chr12 partners were Chr14. Strikingly, in addition to Chr12/Chr14 translocations, another favored fusion partner for Chr14 was Chr13 (3.5% of all metaphases). However, unlike the Chr12/Chr14 translocation, which occurs in 50% of ATM^{-/-} thymic lymphomas,⁶ translocations involving Chr13 were not found amongst 8 thymic lymphomas previously analyzed by spectral karyotyping.⁶ Although we do not currently know the etiology of the Chr13 translocation, our data provide evidence that some high frequency translocation events in primary cells are highly selected for while others are not.

Abnormal V(D)J joining likely accounts for the TCR α/δ translocations found in primary lymphocytes and lymphomas from ATM-deficient patients and mice. In human T cell tumors,

the TCR α joining (J) region is frequently fused with a region 10 Mb centromeric of IgH that includes the TCL1 oncogene locus. The molecular mechanism that leads to generation of DNA breaks downstream of IgH has remained unclear. Although our data do not rule out that stochastic processes or fragile DNA segments can contribute to DNA breakage, we suggest that even breakpoints that are centromeric to the IgH locus might arise from failed V(D)J recombination. In *Atm*^{-/-} thymocytes, unresolved IgH breaks generated by RAG mediated cleavage persist throughout T cell development and are subject to exo-nuclease digestion.^{7,8} These IgH associated breaks with varying degrees of chromosome loss may fuse to the TCR α / δ locus during V α -J α rearrangement. If chromosomal degradation occurs juxtaposing an oncogene on the eroded chromosome to the TCR α enhancer (or other transcription promoting element), deregulation of the transforming gene can ensue, resulting in clonal T cell expansion (Fig. 2). In this way, chromosomal breakpoints centromeric of the IgH locus may be selected for during lymphomagenesis. IgH-TCR α / δ fusions occur at a remarkably high frequency in ATM deficient lymphocytes, but they might also occasionally arise in normal cells because of the high level of DNA damage at these loci and the imperfect nature of the DNA damage checkpoint.^{3,23}

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