

Aberrant V(D)J cleavages in T cell receptor β enhancer- and p53-deficient lymphoma cells

YUN HEE KANG^{1,2}, CHAE-YEON SON¹, CHUL-HO LEE³ and CHUN JEIHYU¹

¹Institute of Bioscience, Department of Bioscience and Biotechnology, Sejong University, Seoul;

²Medical Genomics Research Center, ³Disease Model Research Center, KRIBB, Daejeon, Republic of Korea

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Abstract. Previously, we generated thymic lymphoma cell lines from $E\beta^{R/R}p53^{-/-}$ (EP) double mutant mice where the T cell receptor (TCR) β enhancer ($E\beta$) was deleted, and the p53 gene was inactivated. Here, we characterized the EP cell lines to study the roles of the $E\beta$ and p53 on TCR β rearrangements during lymphomagenesis. Recombination activation genes (RAGs) were expressed, while the TCR β chain was not expressed in the EP cell lines. D β -J β rearrangements were not detected at all, and D β 1 and D β 2 cleavages were also not detected in the EP cell lines. However, J β cleavages suppressed in $E\beta$ mutant thymocytes were readily detected in the EP cell lines. The J β cleavages appeared to be uncoupled, aberrant, RAG-dependent and $E\beta$ -independent and were not detected in a p53 or $E\beta$ single mutant background, suggesting that the J β cleavages are selected in the $E\beta$ and p53 double mutant background. Sequence analysis showed that the cleavage occurred in the cryptic recombination signal sequences (RSSs) present throughout J β gene segments. The results implicate that the uncoupled and aberrant V(D)J cleavages may contribute to double-strand break-mediated genome instability during lymphomagenesis in EP mice.

Introduction

V(D)J recombination is the process of assembling T cell receptor and immunoglobulin genes from V, J and sometimes D gene segments and is the source of the immune system's tremendous diversity of antigen receptors (1,2). V(D)J recombination can be divided into two steps, a cleavage step and a joining step. The initial cleavage step of V(D)J recombination is catalyzed by lymphocyte-specific proteins, recombination activating gene-1 and -2 (RAG-1 and RAG-2,

respectively) which cleave DNA at the junctions between coding sequence and recombination signal sequences (RSSs) to produce double-strand breaks (DSBs) with hairpin coding ends and blunt signal ends (2,3). A subsequent joining step through the non-homologous end joining pathway generates precise signal joints and imprecise coding joints. RSSs are composed of a conserved heptamer (consensus 5'-CACAG TG-3') and nanomer (consensus 5'-ACAAAACC-3') separated by either 12 or 23 base pairs (bp) of varied sequences. Normally, DNA cleavage occurs in a coupled fashion that requires the pairing of 12-bp RSS and 23-bp RSS, although some studies have found that uncoupled cleavages are also possible (4-6). V(D)J recombination occurs in a tightly regulated fashion during lymphocyte development, exhibiting developmental stage, lineage and allele specificity. For example, the complete V β -to-D β J β rearrangement is able to occur in T lymphocytes but not in B lymphocytes where the TCR β locus is also exposed to the same RAG. Thus, although recombination at different TCR and immunoglobulin (Ig) loci is mediated by the same RAG and conserved RSSs, complete rearrangements of TCR genes are limited to T cells, whereas complete rearrangements of Ig genes are limited to B cells. To explain how TCR genes and Ig genes are differentially targeted for recombination by the same enzyme and substrate, Alt *et al* proposed a hypothesis that V(D)J recombination is regulated by modulations in chromatin, which either augment or inhibit accessibility of gene segment to RAGs (7-9). Subsequent studies have shown that V(D)J recombination is regulated by transcriptional *cis* elements such as promoters and enhancers distributed throughout antigen receptor loci, which coordinate changes in the accessibility at gene segments (10-16).

At the TCR β locus, a single TCR β enhancer ($E\beta$) has been described that is located between the C β 2 and V β 14 gene (17). Knockout mouse studies have demonstrated that the $E\beta$ plays an important role in V(D)J recombination of linked TCR β gene segments (11,12,15,16). In the absence of the $E\beta$, there was a severe defect in the formation of D β -to-J β rearrangements, and the entire D β -J β region became hypoacetylated, hypermethylated, and inaccessible to nuclease cleavage (18). The defect was more severe in the V β -to-D β J β rearrangement. Thus, no TCR β was produced and no $\alpha\beta$ T lymphocytes developed in the $E\beta$ -deficient mice (11,12,19). It was also reported that the levels of D β -J β rearrangements were much more severely reduced than the levels of D β and

Correspondence to: Dr Chun Jeih Ryu, Institute of Bioscience, Department of Bioscience and Biotechnology, Sejong University, 98 Gunja-Dong, Gwangjin-Gu, Seoul 143-747, Republic of Korea
E-mail: cjryu@sejong.ac.kr

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J β signal ends in the E β -deficient mice. Based on these observations, the E β was proposed to play a significant role in the joining steps of recombination (15). A detailed study on D β and J β cleavages and rearrangements further showed that E β regulates TCR β rearrangement by promoting accessibility of D β and J β gene segments in CD4⁻CD8⁻ double negative (DN) thymocytes and proper pairing between D β and J β gene segments for joining in CD4⁺CD8⁺ double positive (DP) thymocytes (16). Other studies found E β -independent chromosomal accessibility of D β 1, D β 2 and J β 1 gene segments in DP thymocytes (16,20), suggesting that the accessibility of D β and J β loci can be regulated in an E β -independent manner depending on developmental stages.

In a previous study, we found that E β ^{R/R} thymocytes resulted in the blocking of T cell development at stages where RAG was expressed, and we found that the thymocytes were apoptotic (21). Introduction of a p53 deletion into E β ^{R/R} mice significantly decreased the apoptosis in double mutant thymocytes and accelerated the onset of lethal thymic lymphomas that harbor RAG-dependent chromosome 9, 12, and 14 aberrations (21). However, unexpectedly, TCR β -associated chromosomal aberrations were not observed in the double mutant mice. In this study, we therefore established cell lines from E β - and p53-deficient thymic lymphomas and examined cleavages and rearrangements of D β and J β gene segments in these EP cell lines. We found that aberrant J β cleavages occurred in the EP cell lines and found that the cleavages were uncoupled, RAG-dependent and E β -independent.

Materials and methods

Mice and genotyping. The generation of E β ^{R/R} mice was previously described (16). p53^{-/-} mice were kindly provided by T. Jacks (Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA). E β ^{R/R}p53^{-/-} (EP) mice were generated by mating E β ^{R/R} with p53^{-/-} followed by the mating of F1 heterozygous siblings. Genotyping of E β ^{R/R} and p53^{-/-} were performed by PCR of genomic tail DNA as previously described (16,22). All animal studies were performed according to the institutional guidelines.

Cell lines and flow cytometry. Parts of thymic lymphomas originating from p53^{-/-} or EP mice were minced with forceps in a tissue culture dish. The cells were transferred to a T75 flask in DMEM (Invitrogen, Carlsbad, CA), supplemented with 10% heat-inactivated fetal bovine serum, 0.1 mM 2-mercaptoethanol, antibiotics-antimycotics (Invitrogen) and 2 mM L-glutamine (Invitrogen) and incubated at 37°C in a 5% CO₂ atmosphere. The cells were then diluted 10-fold in the same flask and subsequently subcultured every 2-4 days at a 10- or 20-fold dilution into a new T75 flask. The cell lines were maintained continuously for several months and could be repeatedly frozen and thawed. Two RAG mutant lymphoma cell lines P4980 and P5424 were also maintained as described above (23). For the flow cytometric analysis, cells were incubated with anti-CD4, -CD8 and -TCR β which are direct conjugates from BD Pharmingen (San Diego, CA). Flow cytometry was performed on a FACScalibur apparatus (BD Immunocytometry Systems, San Jose, CA).

Southern and Northern blotting and PCR. Genomic DNA isolation and Southern blotting were performed as previously described (16). D β 1-J β 1, D β 2-J β 2 and C β 1 probes were as previously described (10). All probes were labeled by random priming with ³²P-dCTP. The semi-quantitative nested PCR assays for JAK3, D β 1-J β 1, D β 1-J β 2 and D β 2-J β 2 rearrangements, and the ligation-mediated (LM)-PCR assays for 3'D β 1, 3'D β 2, 5'J β 1, 5'J β 2 and 5'D β 1 cleavages were performed as previously described (16). The LM-PCR assays for 5'D β 2 and 5'JH4 cleavages were also performed as described above. Primer sequences and oligonucleotide probes were as follows: 5'D β 2C, 5'-GTGATATAGATGTTCTCCCAGAGTG-3'; 5'D β 2D, 5'-GACCACATTCAGAACAGGGCTCC-3'; 5'D β 2 probe, 5'-TGCCACCTGGTCTCCCTGCCCTGC-3'; 5'JH4A, 5'-CAGGGTCAGTGACTGTCAAGTTTC-3'; 5'JH4B, 5'-GAGGTGAGGCTGGAATATAGGTCA-3'; 5'JH4 probe, 5'-GATTCTTGTGTGACACCAAGA-3'. PCR products were cloned into pCR2.1-TOPO (Invitrogen) for sequencing. For Northern blot analysis, 10 μ g of total RNA was fractionated on a 1% formaldehyde agarose gel and transferred to a Zeta-probe membrane (Bio-Rad). The RAG1 and RAG2 cDNA probes used in the Northern blotting were isolated from pTet-R1 and pTet-R2 (24). The GAPDH cDNA probe was described elsewhere (21).

Results

Establishment of EP lymphoma cell lines. Previously, we found that introduction of the E β mutation into p53^{-/-} mice dramatically accelerated the onset of lethal thymic lymphomas that harbor RAG-dependent chromosome 9, 12 and 14 aberrations, and all double mutant mice developed terminal thymic lymphomas by 11-17 weeks of age (21). To study TCR β locus rearrangements in the lymphomas, we established thymic lymphoma cell lines from p53^{-/-} and EP mice. Two lymphoma cell lines P99 and P101 were established from p53^{-/-} mice through serial passages in cell culture, which were DP cells that express TCR β (Fig. 1A). Four lymphoma cell lines EP54, EP70, EP116 and EP173 were also established from EP mice through serial passages in cell culture. Most of the EP54, EP70 and EP173 cells were CD8⁺ SP cells, while the EP116 cells were composed of DN (71%) and CD8⁺ SP cells (26%) (Fig. 1A). TCR β signaling has been known to provoke down-regulation of RAG1 and RAG2 expression (25). Consistent with this notion, RAG transcripts were significantly down-regulated in the p53^{-/-} lymphoma cell lines (Fig. 1B). In contrast to the lymphoma cell lines originating from the p53^{-/-} mice, all of the lymphoma cell lines from the EP mice did not express TCR β at all (Fig. 1A, bottom row), and both the RAG1 and RAG2 transcripts were detected in all of the EP cell lines as expected (Fig. 1B).

D β -J β rearrangements in EP cell lines. Previously, we examined RAG activity by assaying for the presence of rearrangements at antigen receptor loci in EP thymic lymphoma cells by Southern blotting (21). We detected IgH and TCR α gene rearrangements, but did not detect TCR β gene rearrangements. As RAG expression was continuously observed in the four EP cell lines (Fig. 1B), we sought to detect D β 1-J β 1, D β 1-J β 2 and D β 2-J β 2 rearrangements in the

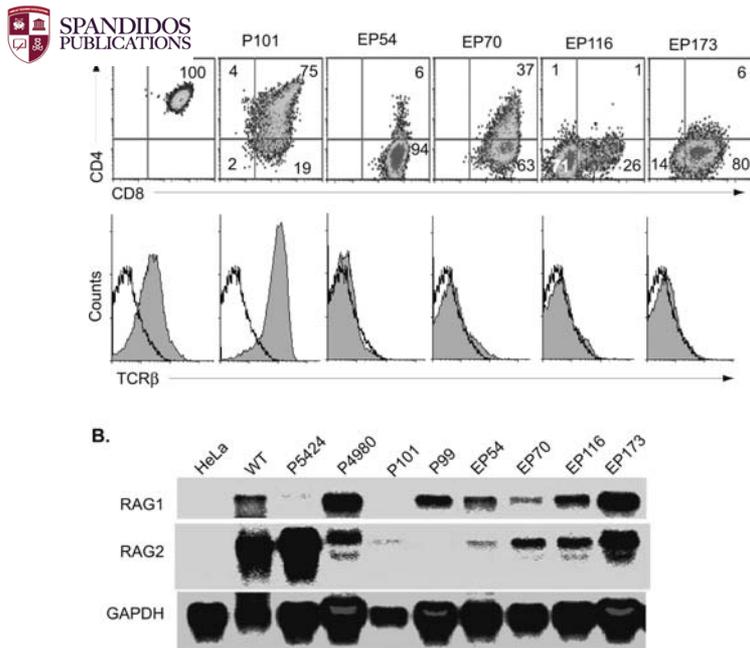


Figure 1. Establishment of thymic lymphoma cells from $p53^{-/-}$ and $E\beta^{R/R}p53^{-/-}$ mice. (A) Two lymphoma cell lines P99 and P101 were established from $p53^{-/-}$ thymic lymphoma tissues, and four lymphoma cell lines EP54, EP70, EP116 and EP173 were established from $E\beta^{R/R}p53^{-/-}$ thymic lymphoma tissues. CD4 and CD8 (upper row), and TCR β staining profiles (lower row) are shown for live cells. Numbers indicate the average percentages of cells in the gated areas. The unfilled population represents TCR β staining in the RAG2-deficient lymphoma cell line P4980, whereas the filled population represents TCR β staining in the indicated lymphoma cell lines. (B) Northern blot analysis of RAG transcripts in total RNAs from the indicated cells. Total RNAs from wild-type thymocytes, P5424 and P4980 cells were included as controls. Hybridization with a GAPDH probe was used to normalize the relative amount of RNA loading in the different lanes.

four EP cell lines by PCR amplification. TCR β gene rearrangements were not detected in the four EP cell lines (Fig. 2). However, DB1-J β 2 and DB2-J β 2 rearrangements were readily detected in 4-week-old lymphoma-free EP mice, although the levels of the rearrangement products were reduced approximately between 25- and 125-fold (16). The results suggest that EP thymocytes whose DB-J β regions undergo rearrangements are removed from the pool, and EP thymocytes whose DB-J β regions remain in the germline configuration are selected during the progression of lymphomagenesis. In contrast, as expected from TCR β expression, clonal TCR β gene rearrangements were detected in the $p53^{-/-}$ lymphoma cell lines (data not shown).

J β cleavages in EP lymphoma cell lines. TCR β rearrangement can be divided into two steps, a cleavage step and a joining step. Although we did not detect any DB-J β rearrangements in the EP cell lines, it does not mean that the DB-J β regions are not accessible to RAG proteins. Previous studies have shown that the E β regulates TCR β rearrangements in both steps depending on the developmental stage (15,16). In DP thymocytes, DB1 and DB2 cleavages were maintained even in the absence of the E β (16). To study whether the DB and J β cleavages are affected in EP cell lines, genomic DNAs were isolated from the lymphoma cell lines and wild-type and

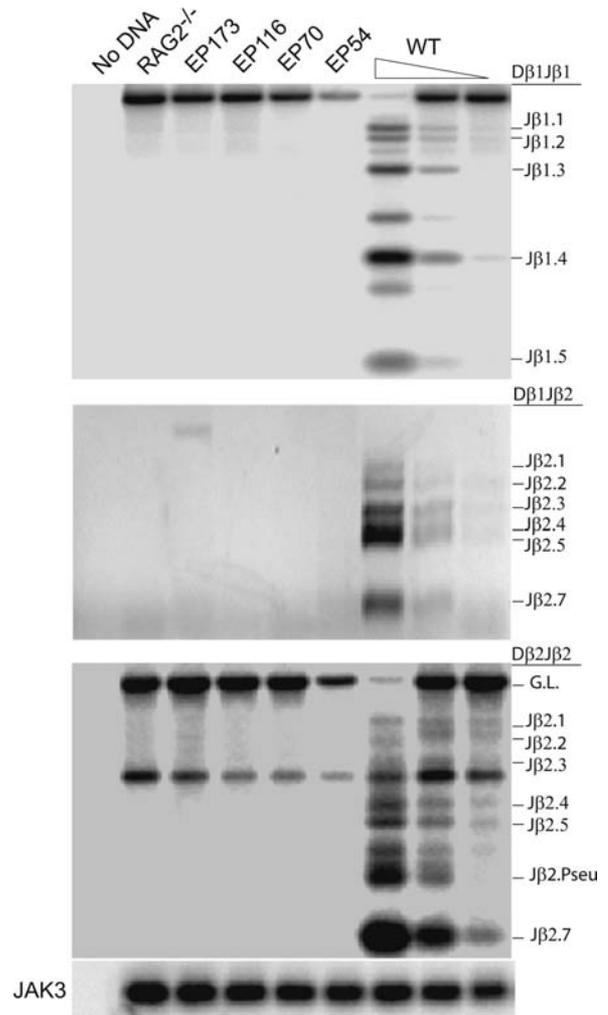


Figure 2. PCR assay for DB1-J β 1, DB1-J β 2 and DB2-J β 2 rearrangements in the EP cell lines. Genomic DNAs were isolated from wild-type thymocytes and EP54, EP70, EP116, and EP173 cell lines. Wild-type DNA was either undiluted or diluted 5-fold with RAG2 $^{-/-}$ kidney DNA. Various rearrangements were measured by a semi-quantitative PCR. JAK3 was amplified to verify DNA quality and relative amount. PCR products were separated on agarose gels and hybridized with specific DB probes. Rearrangements to different J β are labeled. G.L., germline. Representative data from four different experiments are shown.

$E\beta^{R/R}$ thymocytes. DNA cleavages from RAG-mediated V(D)J cleavages were then measured by a semi-quantitative nested LM-PCR (Fig. 3). In wild-type thymocytes, 3'DB1, 3'DB2, 5'J β 1, 5'J β 2, 5'D β 1 and 5'D β 2 cleavages were readily detected (Fig. 3, right). In $E\beta^{R/R}$ thymocytes, 5'J β 1 cleavage was not detected at all, and the levels of 3'D β 1, 3'D β 2 and 5'J β 2 cleavages were also substantially reduced as expected from a previous finding (16). 5'JH4 cleavage was also readily detected in $E\beta^{R/R}$ and wild-type thymocytes, consistent with previous findings (26,27). However, 3'D β 1, 3'D β 2 and 5'JH4 cleavages detected in the $E\beta^{R/R}$ thymocytes were not detected in the EP cell lines (Fig. 3, middle), suggesting that the cells containing the cleavages may be selected against during the progression of lymphomagenesis in EP mice. In contrast to these cleavages, 5'J β 1 and 5'J β 2 cleavage products suppressed in the $E\beta^{R/R}$ thymocytes were readily detected in the EP cell lines (Fig. 3, middle). The J β cleavages were not detected in P5424 and

Table I. Comparison of J β 1, J β 2, DB1 and DB2 cleavage sites in wild-type and E $\beta^{R/R}$ thymocytes and various E $\beta^{R/R}$ p53^{-/-} lymphoma cell lines.

	J β 1	J β 2	DB1	DB2
WT	J β 1.1 CACAGTG (hep) 9X J β 1.2 CACACTA (hep) 1X	J β 2.1 CACAGCA (hep) 5X J β 2.3 CACAGCC (hep) 1X	CACGGTG (hep) 4X CGGTGAT (+2) 1X	CACAATG (hep) 3X
E $\beta^{R/R}$			CACGGTG (hep) 7X	CACAATG (hep) 4X
EP54	J β 1.1CACGGAG (+106) 1X J β 1.2 CACCATA (+228) 3X J β 1.2 CACCTTT (+74) 1X	J β 2.3 CACAGCC (hep) 1X J β 2.1 CACATTC (-71) 1X		
EP70	J β 1.2 CACCCGA (+250) 1X J β 1.2 CGAGGTT (+246) 1X J β 1.2 CACCATA (+228) 1X J β 1.2 CACACCC (+74) 2X	J β 2.3 CACAGCC (hep) 2X J β 2.1 CACATTC (-71) 2X		
EP116	J β 1.1 CACGGAG (+106) 1X J β 1.2 ACTCCAC (+232) 1X J β 1.2 CACCATA (+228) 2X J β 1.2 CACCTTT (+196) 1X J β 1.2 CACACCC (+74) 3X J β 1.3 ACAGACA (+115) 1X	J β 2.3 CACAGCC (hep) 1X		
EP173	J β 1.1 CACGGAG (+106) 1X J β 1.2 CACCTTT (+196) 1X J β 1.2 CACACCC (+74) 2X J β 1.2 CACCAA (+72) 1X J β 1.3 CACTGCA (+369) 1X	J β 2.1 CACAGCA (hep) 1X J β 2.1 AGAATTC (-22) 3X		

The first C of the consensus heptamer of 12-RSS is counted as nucleotide -1. Hep, heptamer; WT, wild-type.

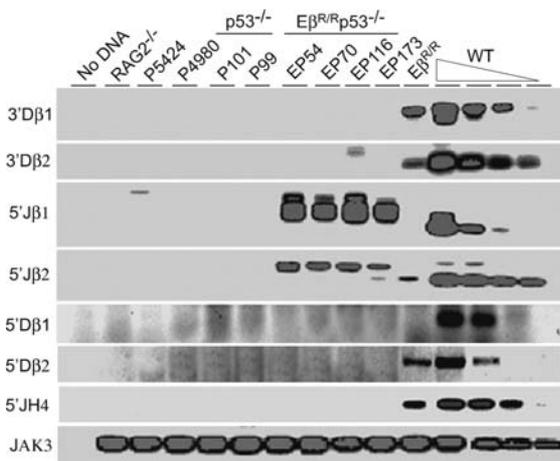


Figure 3. LM-PCR assays for DB, J β and JH4 cleavages. DNAs from the indicated cells were used to assay for DNA cleavages derived from RAG-mediated cleavages at 3'D β 1, 3'D β 2, 5'J β 1, 5'J β 2, 5'D β 1, 5'D β 2 and 5'JH4 by LM-PCR. Wild-type thymocyte DNA was either undiluted or serially diluted every 3-fold, and was included as a control. JAK3 was amplified to verify DNA quality and relative amount. PCR products were separated on agarose gels and hybridized with specific DB1, DB2, J β 1, J β 2 and JH4 probes. Representative data from four different experiments are shown.

P4980 (Fig. 3, left) where parts of the RAG genes were deleted, and were not detected in p53-deficient P99 and P101 where RAG transcripts were significantly down-regulated due to

TCR β expression (Fig. 1). The results suggest that the J β cleavages were E β -independent and RAG-dependent. Furthermore, the J β cleavages appeared to be uncoupled, as 3'D β 1 and 3'D β 2 cleavages were not detected. In the E $\beta^{R/R}$ thymocytes, 5'D β 2 cleavage and non-standard DB1-DB2-J β 2 rearrangements were detected (16). However, 5'D β 1 and 5'D β 2 cleavages and non-standard DB1-DB2-J β 2 rearrangements were not detected at all in the EP cell lines (Figs. 2 and 3), confirming again that the J β cleavages were uncoupled. Taken together, the results suggest that E β -independent and RAG-dependent J β cleavages are uncoupled and are selected in EP cell lines.

J β cleavages occur at cryptic RSSs. The sizes of the J β cleavage products in the EP cell lines were different from those in the E $\beta^{R/R}$ and wild-type thymocytes (Fig. 3). To examine the nature of the cleavages, we sought to clone and sequence the cleavage products from DB1, DB2, J β 1, J β 2, and JH4 cleavages. Twenty-three out of 24 (~96%) cleavage products cloned from wild-type thymocytes started with the consensus heptamers, indicating that the cleavages occurred at the junction between RSSs and coding sequences (Table I). The J β 1 cleavage product was not cloned in the E $\beta^{R/R}$ thymocytes. Twenty-five J β 1 cleavage products were cloned and sequenced in the EP cell lines (Table I), and all of the cleavage products (100%) did not start with the consensus heptamers (the first C of the heptamer of 12-RSS is counted as nucleotide -1).



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cleaved downstream of the original J β 1 RSSs. The cleavage sites were very heterogeneous as 10 different cleavage sites were found in the EP cell lines. Furthermore, 23 out of the 25 (92%) cleavage sites found in the J β 1 gene segments started with 5'-CAC-3' (the first three residues of the consensus heptamer), which indicated that the cleavages occurred at the cryptic RSSs present throughout the J β 1 gene locus (28,29). Although the J β 2 cleavage product was not cloned in the E β ^{R/R} thymocytes, 11 J β 2 cleavage products were cloned from the EP cell lines. Six cleavage products (~55%) were cleaved at the consensus heptamers, and 5 cleavage products were cleaved upstream of the original J β 2.1 RSSs (Table I). The results suggest that the J β 2 cleavages in the EP cell lines were also aberrant, although the percentage of aberrant J β 2 cleavages was not comparable with that of the aberrant J β 1 cleavages. On the other hand, all D β 1 and D β 2 cleavage products (100%) in the E β ^{R/R} thymocytes started with the consensus heptamers (Table I), indicating that the cleavages in the E β ^{R/R} thymocytes occurred at the junction between RSSs and coding sequences. The results indicated that the E β -independent V(D)J cleavages in the EP cell lines were heterogeneous and aberrant, while those in the E β ^{R/R} thymocytes were normal standard cleavages.

Discussion

Many studies have shown that the accessibility of antigen receptor loci to V(D)J recombinase is regulated by transcriptional *cis* elements such as promoters and enhancers distributed throughout antigen receptor loci (10-16). The E β is a single enhancer throughout the TCR β locus and is known to be responsible for the accessibility of D β -J β gene segments. As expected, the control of J β 1 and J β 2 accessibility was reported to be strictly dependent on the E β (30). However, in this study, aberrant J β 1 and J β 2 cleavages were readily detected in the EP cell lines (Fig. 3 and Table I). As the J β cleavages were not detected in the p53^{-/-} lymphoma cell lines and wild-type thymocytes where the E β was normal, the aberrant J β cleavages appear to be suppressed in the presence of the E β . Furthermore, the aberrant J β cleavages appeared to be selected in the absence of p53 since they were not detected in the E β ^{R/R} thymocytes where the p53 gene was normal. Therefore, it is likely that the E β -independent J β accessibility could occur in a certain developmental stage where p53 expression is inactivated. Some studies have also shown that E β -independent J β accessibility increases during DN to DP thymocyte differentiation (20,31), and the DN to DP transition of thymocytes was suggested to be dependent on p53 inactivation (32). As p53 is known as a chromatin accessibility factor and p53-mediated chromosomal accessibility or inaccessibility is possible (33,34), it is possible that J β loci become accessible to RAG-mediated cleavage in the absence of p53 during the progression of lymphomagenesis. We found two putative p53-binding sites in the D β -J β region by using the TFSEARCH program (<http://www.cbrc.jp/research/db/TFSEARCH.html>) and TRANSFEC databases (35). One is between J β 1.2 and J β 1.3, and the other is between D β 2 and J β 2.1. Whether p53 is able to bind two putative binding sites and is able to regulate the accessibility of the D β -J β region remains to be investigated. Furthermore, a recent study

showed that deletion of a small genomic region containing five of the six J β 1 gene segments led to a marked decrease in transcription and rearrangements involving the D β 1 and J β 1.1 gene segments (36), which suggest that transcription and accessibility of J β 1 gene segments can be controlled by an additional *cis*-acting regulatory element. Therefore, it is also likely that E β -independent J β cleavages occur in a certain developmental stage where an additional *cis*-acting element controls accessibility of J β gene segments in the context of p53 inactivation.

In a previous study, introduction of the p53 mutation into E β ^{R/R} mice rescued the thymocytes from apoptosis and allowed the thymocyte development to continue even in the absence of a successful TCR β rearrangement (21). As a consequence, the double mutant thymocytes developed into lethal thymic lymphomas with RAG-mediated chromosome 9, 12, and 14 aberrations (21). In the beginning, introduction of p53 deficiency was expected to allow the persistent presence of unprocessed DNA double-strand breaks (DSB) originating from the TCR β locus during cell proliferation, which would provoke TCR β -mediated chromosomal translocations. It was reported that V(D)J-mediated translocations involving the TCR β and various oncogenes occurred between the D β 1 and cryptic RSSs of various oncogenes in many cases (37). In a large number of cases, a proto-oncogene is juxtaposed to a strong and active regulatory element from antigen receptor loci, and the ectopic/overexpression of the proto-oncogene generally constitutes the initial step of tumorigenesis (37). However, we were not able to detect any TCR β -associated translocations in the EP lymphomas (21), suggesting that translocations involving E β -deleted TCR β loci and oncogenes are not selected during lymphomagenesis. In this study, instead, we found the continued J β cleavages in the EP cell lines (Fig. 3). Most E β ^{R/R} thymocytes undergo massive apoptosis, and the apoptotic cells are partly rescued by p53 deletion (21). However, the EP thymocytes are still susceptible to apoptosis even in the context of a p53 deletion (21). The EP cell lines that we established through this study were also continuously susceptible to apoptosis during serial passages in cell culture (data not shown). Therefore, it is likely that the unprocessed J β cleavages were involved in the p53-independent apoptotic pathway and contributed DSB-mediated genome instability in the EP cell lines. Although we do not have the direct evidence that aberrant J β cleavages are involved in accelerating lymphomagenesis in EP mice, they can still significantly impact genome instability, which may play an important role in T cell tumorigenesis (29,37-40). The correlation between lymphomagenesis and continued persistence of aberrant V(D)J cleavages remains to be investigated. In summary, we found, for the first time, E β -independent V(D)J cleavages in E β and p53 double mutant lymphoma cells. The E β -independent V(D)J cleavages were uncoupled and aberrant and were selected in the p53-deficient background.

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