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No Requirement for V(D)J Recombination in p53-Deficient Thymic Lymphoma

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The p53 tumor suppressor is activated in response to a variety of cellular stress signals, although specific in vivo signals that trigger tumor suppression are unknown. In mouse thymocytes, where p53 inactivation leads to tumorigenesis, several observations suggest that V(D)J recombination of T-cell receptor (TCR) loci could provide a DNA damage signal triggering p53-dependent apoptosis and tumor suppression. Inactivation of p53 would allow V(D)J driven mutation of additional cancer genes, facilitating tumorigenesis. Here, we show that mice with a p53 deficiency in thymocytes and unable to carry out V(D)J recombination are not impaired in the development of thymoma. Recombination-activating gene (RAG) deficiencies were introduced into both $p53^{-/-}$ mice and TgTΔN transgenic mice, a strain in which 100% of the mice develop thymoma due to thymocyte-specific inactivation of p53 by a simian virus 40 T-antigen variant. V(D)J recombination was dispensable for tumorigenesis since thymomas developed with or without the *RAG-1* or *RAG-2* gene, although some delay was observed. When V(D)J recombination was suppressed by expression of rearranged TCR transgenes, 100% of the TgTΔN mice developed thymoma, surprisingly with reduced latency. Further introduction of a RAG deficiency into these mice had no impact on the timing or frequency of tumorigenesis. Finally, karyotype and chromosome painting analyses showed no evidence for TCR gene translocations in p53-deficient thymomas, although abundant aneuploidy involving frequent duplication of certain chromosomes was present. Thus, contrary to the current hypothesis, these studies indicate that signals other than V(D)J recombination promote p53 tumor suppression in thymocytes and that the mechanism of tumorigenesis is distinct from TCR translocation oncogene activation.

The p53 tumor suppressor is activated in response to a variety of cellular stress signals, including DNA damage. Its ability to facilitate growth arrest and/or cell death in response to such signals is believed to be the basis for its tumor suppressor function (see references 5, 16, 22, and 27 for reviews). However, specific in vivo signals that trigger tumor suppression have not been identified. Sixty to eighty percent of the spontaneous malignancies in p53-deficient mice are thymic lymphomas (13, 17), indicating that natural thymocyte events signal p53 tumor suppression. The favored hypothesis is that flawed T-cell receptor (TCR) gene recombination events signal p53-dependent elimination of damaged cells (15, 17, 24, 28). p53 inactivation would thus facilitate the survival of cells carrying tumorigenic mutations. This hypothesis is consistent with several observations, including that (i) double-strand DNA breaks (DSBs) trigger p53 responses (29), (ii) p53 is required for DNA damage-induced thymocyte apoptosis (11, 23), (iii) thymic lymphomas induced by p53 deficiency are clonal, indicating that additional tumorigenic events are required (38), and (iv) in *scid* mice, which accumulate V(D)J breaks, lymphoid malignancies are accelerated by p53 deficiency (15, 28). Since V(D)J translocations are frequently associated with oncogene activation in human and mouse lymphoid tumors, it is reasonable to suspect that these events may be involved in tumorigenesis in the absence of p53-mediated surveillance.

V(D)J recombination affects TCR and immunoglobulin (Ig) DNA rearrangement in developing T and B cells to generate a variety of antigen receptor specificities. Normally this process occurs during specific stages of T- and B-cell differentiation to yield a single productive rearrangement per cell for each polypeptide component of the receptor. The initiating event in V(D)J recombination, the generation of specific DSBs, requires two recombination activating genes (RAGs), *RAG-1* and *RAG-2* (9, 31). Mice deficient in either of these genes fail to undergo V(D)J recombination and are immunodeficient due to the lack of mature T and B cells (25, 37). Thus, these mice provide an approach for assessing the role of V(D)J recombination in thymomagenesis associated with p53 deficiency. Here we examine the impact of inactivating V(D)J recombination on tumorigenesis by introducing RAG deficiencies and/or rearranged TCR transgenes into mice with a thymocyte p53 deficiency. Additionally, we analyze the chromosomes of p53-deficient thymomas for evidence of TCR translocations and other aberrations.

MATERIALS AND METHODS

Mice. RAG-$1^{-/-}$ (C57BL/6J-sv/129), *scid*/*scid* (C57BL/6J), TgN(TcrLCMV) (B6D2), and $p53^{-/-}$ (C57BL/6J) mice were from Jackson Laboratory, and RAG-$2^{-/-}$ mice (129, SvEv) were from Taconic Laboratory. TgTΔN mice (B6D2), previously referred to as TgLST1135, abundantly express the *dl*1135 simian virus 40 (SV40) large T antigen (T-Ag) in thymocytes under the regulation of the lymphotropic papovavirus transcriptional signals (38). Although low levels of the transgene are also expressed in B cells, only thymic lymphoma develops in these mice (38). The *dl*1135 protein (referred to here as TΔN) binds p53 and the pRB family proteins and inactivates p53 in vivo (10, 24). TΔN is defective in transformation of cultured cells due to a deletion (residues 17 to 27) that inactivates an N-terminal transformation function (32). The TΔN transgene harbors a deletion in the small t antigen splice donor site and does not express SV40 small t antigen. With the exception of *scid* mice, PCR assays of tail DNA were used to

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screen mouse genotypes as previously described (17, 38) or according to Jackson Laboratory [<http://www.jax.org/resources/documents/imr/protocols/TgN(Tcr-LCMV).html>]. *RAG-1* alleles were identified by using two primer pairs: for *RAG-1* wild type, RAG-1F (5′-CCA GTA GAT ACC ATT GCG AAG AGG-3′) and RAG-1B (5′-CAC GTT CTG TGA ACC ATG CTC TAT C-3′); and for *RAG-1* knockout, RAG-1B and Neo-R (5′-CCG CTT CCA TTG CTC AGC GG-3′). PCR conditions were 35 cycles of 30 s at 94°C, 1 min at 59°C, and 1 min at 72°C. *RAG-2* alleles were identified by using two primer pairs: for *RAG-2* wild type, RAG-2A (5′-GGG AGG ACA CTC ACT TGC CAG TA-3′) and RAG-2B (5′-AGT CAG GAG TCT CCA TCT CAC TGA-3′); and for *RAG-2* knockout, RAG-2B and Neo-2 (5′-AGG TGA GAT GAC AGG AGA TC-3′). PCR conditions were 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. The *scid* mice were screened by using enzyme-linked immunosorbent assay (ELISA) detection of serum IgM, which is absent in *scid* mice (14).

To generate TgTΔN or $p53^{-/-}$/$RAG^{-/-}$ and TgTΔN or $p53^{-/-}$/$RAG^{+/-}$ mice, TgTΔN or $p53^{-/-}$ mice were bred with $RAG^{-/-}$ (RAG-1 or RAG-2) mice, and F_1 offspring were intercrossed. $p53^{-/-}$ mice were bred to C57BL *scid*/*scid* mice, and F_1 offspring were intercrossed to derive $p53^{-/-}$ *scid*/*scid* and $p53^{-/-}$ *scid*/wt (wild-type) animals. The latter were distinguished from $p53^{-/-}$ wt/wt mice, which are both positive by ELISA, in test breeding with *scid*/*scid* mice. If heterozygous at the *scid* locus, ~50% of the offspring scored negative by ELISA (*scid*/*scid*). A similar breeding strategy was used to generate TgTΔN/*scid*/*scid* and TgTdN/*scid*/wt mice. To generate TgTΔN/TgTcr/RAG-$1^{-/-}$ and TgTΔN/TgTcr/RAG-$1^{+/-}$ mice, TgTΔN/RAG-$1^{-/-}$ mice were crossed to TgN(TcrLCMV) mice, and F_1 TgTΔN/TgTcr/RAG-$1^{+/-}$ mice were further bred with TgTΔN/RAG-$1^{-/-}$ mice. Animals were sacrificed and necropsied upon showing distressed breathing due to extensive thymic enlargement or were autopsied upon death. Fresh tumor samples were divided and either fixed for immunohistochemistry analysis or used for thymocyte isolation.

FACS analysis. Thymoma cell type was determined by fluorescence-activated cell sorting (FACS) analysis using phycoerythrin (PE)–anti-CD90 (Thy-1.2) and fluorescein isothiocyanate (FITC)–anti-CD45R/B220 antibodies (PharMingen), and thymocyte distribution was determined by using FITC–anti-CD4 and PE–anti-CD8 antibodies (PharMingen), as described previously (18). The thymocyte proliferation index was determined by in vivo labeling with bromodeoxyuridine (BrdU) (Boehringer Mannheim) for 1 h after intraperitoneal injection (0.1 ml/10 g of body weight), isolation of thymocytes as described previously (24, 38), and FACS analysis with FITC–anti-BrdU antibody (Boehringer Mannheim) and propidium iodide staining. The total thymocyte apoptotic index was determined by FACS using a modification of the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling assay (Cell Death Detection kit) as recommended by Boehringer Mannheim.

Karyotype and fluorescence in situ hybridization analyses. Minced mouse thymoma cells were harvested directly, using routine cytogenetics laboratory procedures for preparation of metaphase chromosomes (41). Karyotypes were determined by standard Giemsa banding techniques (36). Biotinylated mouse whole chromosome paint probes for chromosomes 6, 13, and 14 were used as instructed by the supplier (Oncor, Inc., Gaithersburg, Md.). These probes were obtained from flow-sorted mouse chromosomes and were optimized to hybridize only to target chromosomes. Hybridization to repetitive sequences was suppressed by including excess mouse repetitive DNA. Biotinylated probes were detected by incubation with rabbit antibiotin antibody, biotinylated anti-rabbit antibody, and streptavidin-FITC. Mouse chromosomes were counterstained with propidium iodide. Images of metaphases were captured by a charge-coupled device camera and analyzed with Smartcapture software (Vysis Inc., Downers Grove, Ill.).

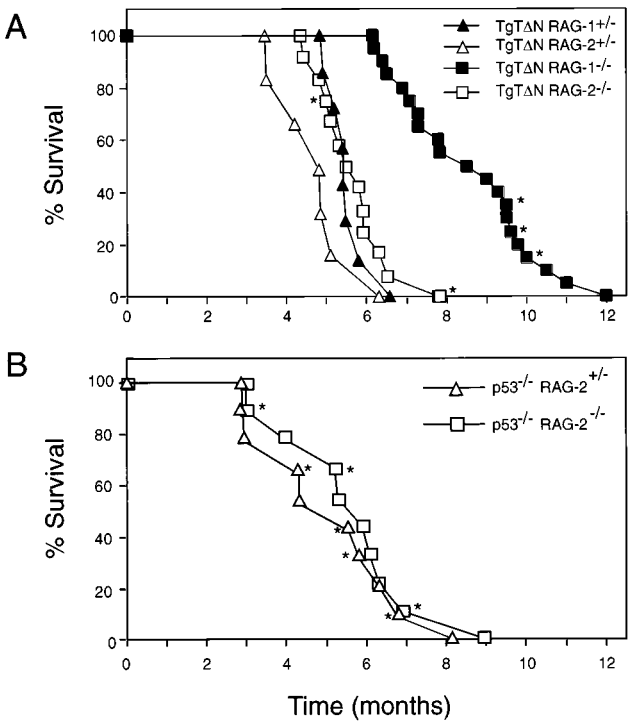

FIG. 1. Thymoma develops in TgTΔN and $p53^{-/-}$ mice with altered V(D)J recombination. Survival of mice is plotted against time. Each data point reflects the sacrifice or death of a terminally ill mouse. Due to strain differences, littermates of each cross serve as controls. Mice that did not show overt thymoma are indicated by asterisks. (A) Effect of a RAG-deficient background on the timing and frequency of thymomagenesis in TgTΔN mice. As with TgTΔN mice (38), all of the seven TgTΔN/RAG-$1^{+/-}$ and all of the six TgTΔN/RAG-$2^{+/-}$ mice developed thymoma. The shorter latency in TgTΔN/RAG-$2^{+/-}$ mice is most likely caused by background strain effects. Overt thymoma developed in the majority of TgTΔN/RAG-$1^{-/-}$ (85%, $n = 20$) and TgTΔN/RAG-$2^{-/-}$ (83%, $n = 12$) mice. Three mice of the RAG-1-deficient set and two mice of the RAG-2-deficient set showed no overt thymoma at the time of sacrifice. One TgTΔN/RAG-$1^{-/-}$ mouse developed a lymph node tumor, and one TgTΔN/RAG-$2^{-/-}$ mouse had splenomegaly in addition to an enlarged thymus. No neoplasm was detected in the other three mice, a phenotype also observed in mice with only a RAG deficiency (not shown). Six TgTΔN/RAG-$2^{-/-}$ mice are not included in the survival analysis since their early death precluded assessment of thymoma. Of these, three had lymph node tumors of an undetermined cell type. (B) Effect of RAG-2 deficiency on $p53^{-/-}$ mice. Thymoma developed in 56% of the nine $p53^{-/-}$/RAG-$2^{+/-}$ mice and in 67% of the nine $p53^{-/-}$/RAG-$2^{-/-}$ mice. The remaining mice developed other characteristic $p53^{-/-}$ tumors, including sarcoma, testicular tumors, and lymphoma (13, 17).

RESULTS

Mice deficient in both RAG and p53 develop thymoma. We introduced RAG-1 or RAG-2 deficiency into mice lacking thymocyte p53 activity by using standard genetic crosses as outlined in Materials and Methods. Since T-cell tumorigenesis in $p53^{-/-}$ mice is not fully penetrant and other complicating tumor types develop, in most experiments we used transgenic mice that express an SV40 T-Ag derivative, TΔN, specifically in thymocytes (see Materials and Methods). This oncoprotein inactivates p53, and we previously showed that 100% of TgTΔN mice develop exclusively thymic lymphoma at about 5 months of age (38). Prior to 2 months of age, thymocyte distribution is normal and consists predominantly of CD4 CD8 double-positive (DP) thymocytes (38). From 2 to 5 months, the thymus expands rapidly and consists increasingly of a single Vβ T-cell class high in cell surface CD3 and CD4 CD8 single positive, double negative, or DP (38). This phenotype is dependent on a functional T-Ag p53-binding domain (40), and T-cell tumors are indistinguishable from those induced by p53 gene inactivation (22a, 38). Furthermore, preneoplastic TgTΔN thymocytes are defective in irradiation-induced p53-dependent apoptosis (24).

The high frequency and predictability of thymic lymphoma in TgTΔN mice facilitate quantitative studies on the role of V(D)J recombination. However, in addition we generated $p53^{-/-}$ mice deficient in RAG-2 to control for any unexpected effects of TΔN. If V(D)J recombination is required for T-cell tumorigenesis in the absence of p53, inactivation of this process would inhibit tumorigenesis. TgTΔN/RAG-$1^{-/-}$, TgTΔN/RAG-$2^{-/-}$, and $p53^{-/-}$/RAG-$2^{-/-}$ mice were generated through standard backcrosses, and the timing and frequency of thymoma were measured. All of the mice became ill and were sacrificed and necropsied (Fig. 1A). Thymic lymphoma had developed in the majority of RAG-deficient TgTΔN mice, although the frequency was somewhat reduced (85% in TgTΔN/RAG-$1^{-/-}$ mice and 83% in TgTΔN/RAG-$2^{-/-}$ mice [Table 1]). A few mice developed lymph node tumors or had no

TABLE 1. Summary of effect of V(D)J recombination inactivation on p53-deficient thymoma

p53 inactivation	Control locus	V(D)J inactivation	T-cell maturation	Thymoma frequency (%)	Survival time (t_{50}, days)
TΔN	RAG-1$^{+/-}$		Yes	100	165
TΔN		RAG-1$^{-/-}$	No	85	238
TΔN	RAG-2$^{+/-}$		Yes	100	146
TΔN		RAG-2$^{-/-}$	No	83	167
p53$^{-/-}$	RAG-2$^{+/-}$		Yes	55	165
p53$^{-/-}$		RAG-2$^{-/-}$	No	67	180
TΔN	RAG-1$^{+/-}$	TgTcr	Yes	100	110
TΔN		TgTcr + RAG-1$^{-/-}$	Yes	100	101
TΔN	*scid*/wt		Yes	93	183
TΔN		*scid*/*scid*	No	62	210
p53$^{-/-}$	*scid*/wt		Yes	60	183
p53$^{-/-}$		*scid*/*scid*	No	0	214

obvious neoplasm (a phenotype also observed in mice with RAG deficiency alone [see the legend to Fig. 1]). Thymoma developed in 55% of the p53$^{-/-}$ RAG-2$^{+/-}$ mice (Fig. 1B), a frequency lower than for TgTΔN mice due to the development of other tumor types (as previously observed). Inactivation of V(D)J recombination did not reduce this frequency, as 67% of p53$^{-/-}$ RAG-2$^{-/-}$ mice developed thymoma (Fig. 1B; Table 1). FACS analysis of three TgTΔN/RAG-1$^{-/-}$ thymomas showed that cells were CD4 CD8 DP, CD3 negative, and Vβ negative, consistent with the fact that V(D)J recombination is inactive (data not shown). The high frequency of thymoma in p53 and RAG-deficient mice indicates that V(D)J recombination, and T-cell differentiation in general, is dispensable for T-cell tumorigenesis.

Although most RAG-deficient TgTΔN mice developed thymoma, tumors arose more slowly or with longer latency, based on extended survival (Fig. 1A; Table 1). All of the control TgTΔN/RAG-1$^{+/-}$ mice developed thymoma with a t_{50} (time at which half of the animals were sacrificed or died due to overt illness) of 165 days, similar to that previously reported for TgTΔN mice (38). The t_{50} for TgTΔN/RAG-1$^{-/-}$ mice, however, was 238 days. Thymoma developed with reduced latency in control TgTΔN/RAG-2$^{+/-}$ mice (t_{50} = 146 days) compared to TgTΔN mice, most likely due to a background strain effect. Relatively, in TgTΔN/RAG-2$^{-/-}$ littermates the t_{50} was again delayed (167 days), albeit to a lesser extent. A similar delay was also observed in p53$^{-/-}$ RAG-2$^{-/-}$ mice (t_{50} = 180 days) compared to p53$^{-/-}$ RAG-2$^{+/-}$ mice (t_{50} = 165 days; Fig. 1B, Table 1).

V(D)J recombination is not required for T-cell tumorigenesis. Since RAG-deficient mice harbor fewer thymocytes and are blocked in T-cell development (25, 37), the observed delay and reduced frequency of thymoma may simply reflect a smaller and/or altered target cell population rather than a direct effect of V(D)J recombination. This could cause a dramatic effect, particularly when p53 is inactivated as a result of transgene expression, which may not occur in every cell. To circumvent these complications, T-cell development was rescued in TgTΔN/RAG-1$^{-/-}$ mice by thymocyte-specific expression of rearranged TCRα and TCRβ transgenes (9). Crosses with mice harboring lymphocytic choriomeningitis virus-specific TCR transgenes [TgN(TcrLCMV)] (33) were carried out as described in Materials and Methods. The effect of TCR transgenes on thymomagenesis in control TgTΔN/TgTcr/RAG-1$^{+/-}$ mice also served as a test for the role of V(D)J recombination, in that expression of rearranged TCR transgenes alone suppresses (though does not eliminate) V(D)J recombination (42). Surprisingly, tumorigenesis was accelerated in TgTΔN/TgTcr/RAG-1$^{+/-}$ mice (t_{50} = 110 days) relative to TgTΔN/RAG-1$^{+/-}$ littermates (t_{50} = 165 days) (Fig. 2). Hence, suppressed V(D)J recombination provided no deterrent to tumorigenesis. The reason for accelerated tumor development is unknown, but it could reflect increased target cell numbers or altered thymocyte proliferation. In support of this possibility, young (6-week-old) TgTΔN/TgTcr/RAG-1$^{+/-}$ mice had an abnormally high percentage of thymocytes in S phase (22.9%), while nontransgenic and TgTΔN S-phase thymocytes comprised only 8.8 and 8.9%, respectively, of the total population. Total apoptosis levels were unchanged (data not shown).

To measure the effect of complete inactivation of V(D)J recombination, TgTΔN/TgTcr/RAG-1$^{+/-}$ mice were further backcrossed to RAG-1$^{-/-}$ mice (see Materials and Methods). Importantly, RAG deficiency had no impact on the timing or frequency of tumorigenesis in TgTΔN/TgTcr mice (Fig. 2). All TgTΔN/TgTcr/RAG-1$^{-/-}$ mice developed thymic lymphoma within the same accelerated time frame (t_{50} = 101 days) as TgTΔN/TgTcr/RAG-1$^{+/-}$ mice. The predominant cell type in each of three thymomas was CD4 CD8 DP, CD3 positive, and TCRβ positive. This result shows that the increased latency and slightly reduced frequency of thymoma in RAG-deficient TgTΔN mice was not due to the absence of V(D)J recombination. Since tumorigenesis was unaffected by complete absence of V(D)J recombination in the presence of T-cell maturation, the recombination process is not required for, nor

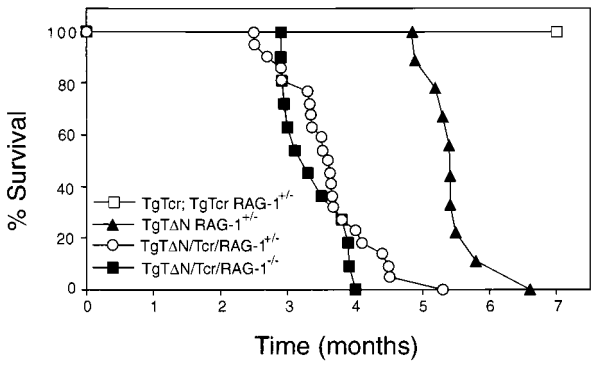

FIG. 2. Effect of TCR transgene expression on the timing and frequency of thymomagenesis. TgTΔN/TgTcr/RAG-1$^{+/-}$ mice (n = 22) developed thymoma with reduced latency compared with TgTΔN/RAG-1$^{+/-}$ mice (n = 9). RAG-1 deficiency (n = 11) had no further effect. All mice developed thymoma.

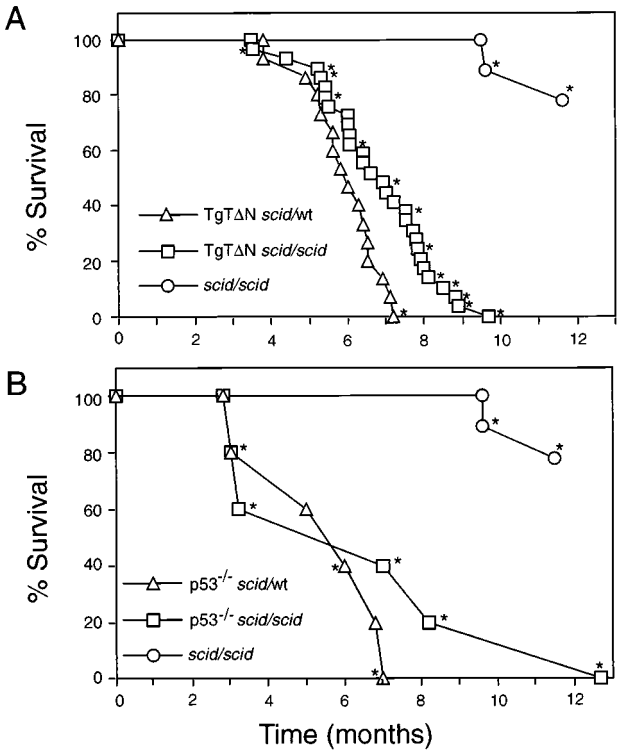

FIG. 3. Tumorigenesis is not accelerated in *scid* mice. The effect of the *scid* mutation on the timing and frequency of thymomagenesis in TgTΔN and $p53^{-/-}$ mice was measured as described in the legend to Fig. 1. (A) TgTΔN/*scid*/*scid* mice ($n = 29$) developed thymoma with increased latency compared with their TgTΔN/*scid*/wt littermates ($n = 15$); 93% of the TgTΔN/*scid*/wt mice and 62% of the TgTΔN/*scid*/*scid* mice developed thymoma. One TgTΔN/*scid*/wt mouse and four TgTΔN/*scid*/*scid* mice developed splenomegaly. No neoplasm was detected in seven TgTΔN/*scid*/*scid* mice. (B) Thymoma developed in 60% of the five $p53^{-/-}$/*scid*/wt mice, but in none of the five $p53^{-/-}$/*scid*/*scid* mice. The nine control *scid*/*scid* mice did not develop thymoma, and most remained alive for the duration of the study. Mice without overt thymoma are indicated by asterisks.

TABLE 2. Chromosome aberrations in p53-deficient thymic lymphomas[a]

Tumor	Chromosome(s) with tumor-specific duplications (frequency)	Translocation(s) (frequency)	Chromosome no.	
			Avg	Range
TgTΔN-1	4 (9/10); 5, 11, and 15 (8/10)		47	40–49
TgTΔN-2	15 (7/8)	[t(5;?) (2/8)]	44	40–48
TgTΔN-3		[t(5;5) (3/5)]	41	38–41
TgTΔN-4	9 (4/5); 4 and 13 (5/5)	[t(5;5) (1/5)]	47	42–49
TgTΔN-5			42	40–46
TgTΔN-6	[5 (3/5)]		41	39–41
TgTΔN-7	[5 and 19 (2/5)]	[t(x;?) (1/5)] and [t(2;14) (1/5)]	41	40–42
TgTΔN-8			39	39–41
TgTΔN-9	1, 3, 8, (4/5); 4, 5, 10, and 15 (5/5)		51	50–53
$p53^{-/-}$-1	1, 3, 4, 17, and 19 (5/5)		49	48–50
$p53^{-/-}$-2	4 (7/10)	t(15;15) (8/10)	42	39–60
$p53^{-/-}$-3		[t(2;19) (5/10)]	39	39

[a] Trisomic chromosomes and translocations present in the majority of metaphases (≥70%) are listed; less frequent chromosome duplications are in brackets. The frequencies of the aberrations are indicated in parentheses as number of cells with the aberration/total cell number examined. Sample TgTΔN-8 contained double minute chromosomes. Average chromosome numbers are rounded to the nearest whole integer. The ranges of chromosome numbers in individual cells are also listed.

does it affect the rate of, T-cell tumorigenesis induced by p53 inactivation.

No acceleration of T-cell tumorigenesis in a *scid* background. Although the foregoing studies show that V(D)J recombination is dispensable for thymoma development, it is possible that the recombination pathway, if present, plays a role that may be compensated for in its absence. This possibility seems unlikely given that the putative compensatory process would not rescue V(D)J recombination and would thus function by a different mechanism. Nonetheless, two approaches were used to test this possibility. In the first approach, tumorigenesis rates were tested in the *scid* background (8). *scid* mice are defective in the DNA-dependent protein kinase activity required for rejoining of V(D)J coding DNA ends (12, 43). As a result, *scid* lymphocytes accumulate V(D)J-associated DSBs. Thus, although the recombination overall is inhibited, the hypothesized signal to p53 would be increased. If such breaks provide secondary cooperating mutations for tumorigenesis in the absence of p53, thymoma development would be accelerated in this background. Indeed, previous studies showed that $p53^{-/-}$/*scid* mice died earlier than $p53^{-/-}$ littermates (7, 15, 28). However, because p53 was absent from all cells, these mice developed B-cell, as well as T-cell, lymphoma. In TgTΔN mice, exclusive analysis of thymoma is possible. Consistent with results for the TgTΔN/$RAG^{-/-}$ mice, development of thymic lymphoma was not accelerated in TgTΔN/*scid* mice (Fig. 3A; Table 1). Instead, tumorigenesis was slightly delayed in these mice ($t_{50} = 210$ days) compared to TgTΔN/*scid*/wt mice ($t_{50} = 183$ days). To determine whether this effect was unique to the transgenic mice, $p53^{-/-}$/*scid* crosses were performed (Fig. 3B). Again, thymomagenesis was clearly not accelerated. Although three of five $p53^{-/-}$/*scid*/wt mice developed thymoma within the usual time frame ($t_{50} = 183$ days), none of the $p53^{-/-}$/*scid*/*scid* mice developed thymoma. Rather, these mice died of other causes with a t_{50} of 214 days (see the legend to Fig. 3). Because the number of $p53^{-/-}$/*scid*/*scid* mice analyzed was small, this difference may not be statistically significant. However, lack of thymoma acceleration is consistent with what occurs in the TgTΔN/*scid*/*scid* mice. The difference in these experiments compared with those reported previously is not clear but may be attributable to the difference in *scid* background strains used (see Discussion). The *scid*-dependent delay observed here in TgTΔN mice most likely reflects a reduced number of thymocytes as in RAG-deficient mice. Thus, these experiments show no evidence that increased V(D)J DSBs accelerate T-cell lymphoma development in the absence of p53.

Chromosome analysis of p53-deficient thymomas. As another test for participation of V(D)J recombination in p53-deficient thymic lymphoma, chromosomes of TgTΔN thymomas were analyzed for evidence of translocations involving TCR loci. If flawed recombination events provide the signal for p53 tumor suppression, these events should contribute to tumorigenesis in the absence of p53 and would be identifiable in tumor cells. This is a reasonable expectation since tumorigenic translocations involving TCR or Ig genes occur in several human T- and B-cell neoplasms (20, 30, 35). Furthermore, TCR translocations are readily observed with increased frequency in ataxia telangiectasia (AT) lymphoid malignancies (20, 39) and in thymomas induced by ATM (AT mutated) deficiency in

TABLE 3. Proto-oncogenes encoded by tumor-specific trisomies[a]

Chromosome no.	Proto-oncogenes
1	*elk4*, K-*ras1*, L-*myc2*, *mdm4*, *mybl1*
4	*fgr*, *jun*, *lci*, *lck*, L-*myc1*, *lyn*, *mos*, *mpl*, *ski*, *lag*, *ril2*, *scl/tal1*
5	*kit*, *gro1*, L-*trm3*, N-*myc2*
15	*myc*, *ril1*

[a] Information was derived from the MRC Database of the Mammalian Genetics Unit at Harwell, England (http://www.mgu.har.mrc.ac.uk/genelist/genelists.html). *ril1* and *ril2*, radiation-induced leukemia 1 and 2; *lag*, leukemia-associated gene.

TABLE 4. Painting analysis of TCR chromosomes[a]

Mouse genotype no.	Aberration
TgTΔN-10	—
TgTΔN-11	+6 [3]/+13 [1]/+14 [2]
TgTΔN-12	+6 [6]/+13 [3]/+14 [4]
TgTΔN-13	—
TgTΔN-14	—
TgTΔN-15	+6 [1]/+6+6 [2]/+13 [3]
TgTΔN-16	+6 [2]/+13+13 [1]
TgTΔN/RAG-2$^{+/-}$	+13 [1]/+14+14 [2]
TgTΔN/Tcr/RAG-2$^{-/-}$-1	+6 [1]/+14 [1]/+14+14 [2]
TgTΔN/Tcr/RAG-2$^{-/-}$-1	—
ATM$^{-/-}$	t(14;14) [20]

[a] Twenty cells for each thymoma specimen were examined by using painting probes for chromosomes 6, 13, and 14 as described in Materials and Methods. Shown are cells with trisomies, tetrasomies, or translocations (number of metaphases in brackets). No listing for a given chromosome indicates disomy. —, samples where all 20 metaphases were disomic for each chromosome.

mice (4, 45). Evidence suggests that ATM has a role in some p53 DNA damage-induced checkpoints (19).

Karyotypes of nine TgTΔN and three p53$^{-/-}$ terminal thymomas were analyzed (Table 2). Only three tumors harbored translocations in the majority of cells analyzed, and none of these involved the TCR-bearing chromosomes 6, 13, and 14. In contrast, aneuploidy, including abundant trisomy and some tetrasomy, was frequently observed. Although any of the 20 chromosomes could be affected, trisomy of chromosome 1, 4, 5, or 15 was frequently present (Table 2). Such specificity in chromosome aberrations may indicate the participation of oncogene amplification in thymoma development. Although these data are not sufficient to address the role of any given oncogene, several genes, including some (c-*myc* [2, 6], *lck* [1], and *scl/tal1* [3, 21]) known to play a role in T-cell tumorigenesis, are present on the selected chromosomes (Table 3).

To be sure that karyotype analysis did not overlook TCR translocations not visibly detectable, we further analyzed tumors by chromosome painting. Metaphase spreads from seven

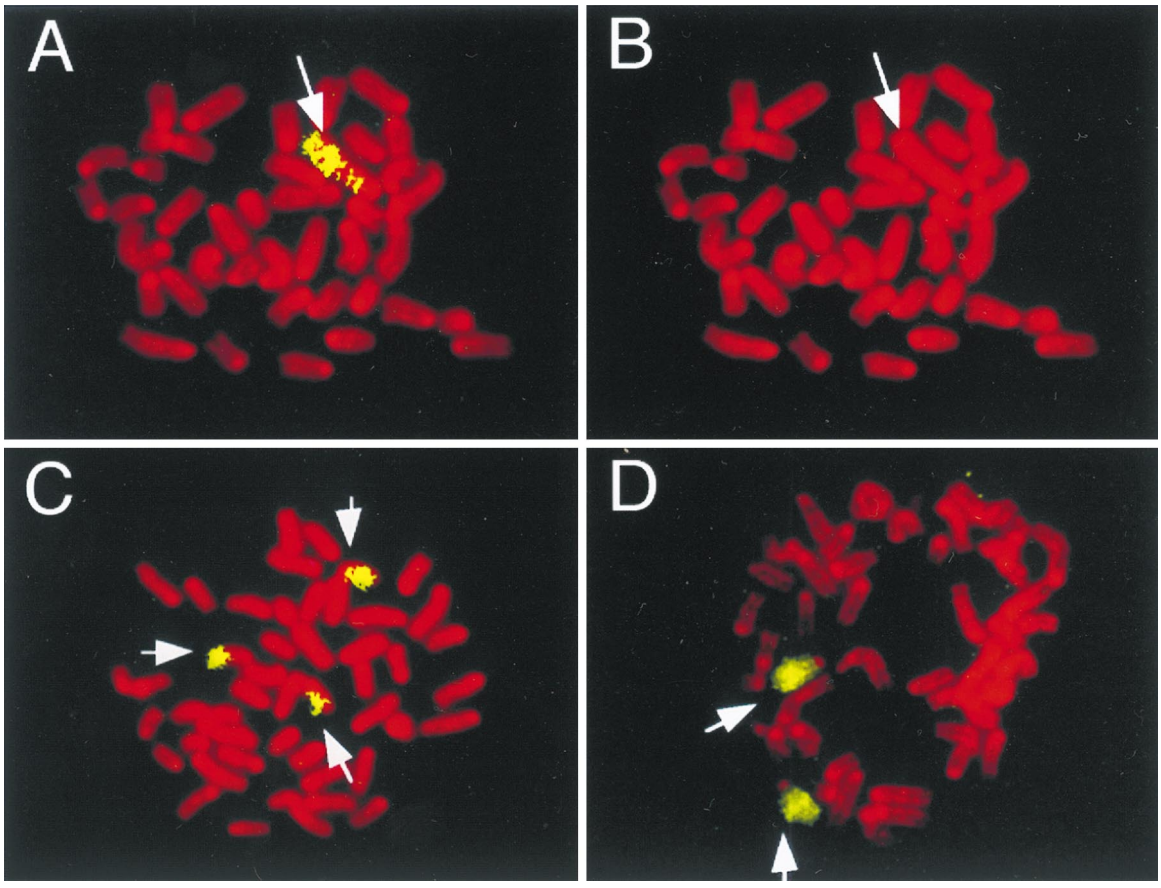

FIG. 4. p53-deficient thymomas do not contain TCR translocations. Metaphase spreads were hybridized with paint probes as indicated in Materials and Methods. (A) Chromosome 14 probe showing painting of one large chromosome illustrating t(14;14) in an ATM$^{-/-}$ control thymoma. (B) The same metaphase using only a filter for propidium iodide. (C) Trisomy 14 in a TgTΔN-3 thymoma, representative of trisomy observed in several samples (Table 4). (D) Representative disomy 14 in a TgTΔN-1 thymoma. Note that the painting probes do not paint the entire chromosome because of suppression by total mouse repetitive sequences to maintain specificity (34).

TgTΔN, one TgTΔN/RAG-$1^{+/-}$, and two TgTΔN/RAG-$1^{-/-}$ tumors were painted with whole-chromosome probes for chromosomes 6, 13, and 14. None of these tumors showed evidence of TCR translocation, while a control ATM-deficient tumor showed a t(14;14) translocation in all cells examined (Fig. 4; Table 4). Together with the genetic evidence described above, these studies indicate that V(D)J recombination does not participate significantly in p53-deficient thymomagenesis. The chromosome analysis also suggests that the mechanism for thymic lymphoma induction may be different for p53 deficiency than for ATM deficiency, in which case these factors function in distinct pathways in thymocyte tumor suppression. This notion is supported by the observation that thymoma development is more rapid in $ATM^{-/-}/p53^{-/-}$ mice than in mice harboring either single deficiency (44).

DISCUSSION

Although previous data are consistent with the hypothesis that TCR gene recombination could be involved in p53-deficient T-cell tumorigenesis, studies presented here indicate that this process is not required. Inactivation of V(D)J recombination using RAG-deficient mice did not inhibit T-cell tumorigenesis predisposed by p53 deficiency. This was most evident in TgTΔN/RAG-$1^{-/-}$ mice in which T-cell maturation was rescued by expression of rearranged TCRα and -β transgenes. In that case, neither the timing nor the frequency of tumorigenesis was affected by the absence of V(D)J recombination relative to controls. Interestingly, expression of rearranged TCR genes in TgTΔN mice accelerated T-cell tumorigenesis. Although initially surprising, the high S-phase thymocyte population associated with TCR transgene expression could account for this result. Neither $p53^{-/-}$ nor TgTΔN thymocytes proliferate above normal levels prior to overt thymoma, while thymoma cells have a high proliferative index (22a). Thus, proliferation of TCR transgene-expressing thymocytes may overcome the need for genetic events that stimulate this step in tumorigenesis. Regardless of the reason for acceleration of these tumors, they do not require V(D)J recombination. Thymoma developed in 100% of TgTΔN/TgTcr mice, even though allelic exclusion substantially suppresses recombination. Moreover, further inactivation of V(D)J recombination by RAG deficiency had no effect on thymoma development. Recent studies by Nacht and Jacks (27) also concluded that V(D)J recombination was not required for p53 deficiency-induced thymoma. Their studies showed that a RAG deficiency in the $p53^{-/-}$ background did not significantly alter tumor development in $p53^{-/-}$ mice.

In the absence of TCR transgene expression, RAG deficiency alone caused a delay in thymoma development, although these tumors occurred with a high frequency. This effect was most dramatic in TgTΔN/RAG-$1^{-/-}$ mice, which lived 44% longer than controls. The effect was not as dramatic in TgTΔN/RAG-$2^{-/-}$ or $p53^{-/-}$/RAG-$2^{-/-}$ mice, although these mice lived longer (14 and 9%, respectively) than controls as well. In the study by Nacht and Jacks (27), a RAG-1 deficiency did not significantly alter the survival time of $p53^{-/-}$ mice. Thus, the delay we observed in TgTΔN/RAG-$1^{-/-}$ mice may be specific to the dependence on transgene expression for p53 inactivation. The altered thymocyte population caused by RAG deficiency could affect the percentage of cells expressing TΔN. While we know that the majority of TgTΔN thymocytes express TΔN, we did not examine the TgTΔN/RAG-$1^{-/-}$ thymocytes for TΔN expression. Our experiments using TCR transgenic mice support the conclusion that the delay in thymoma development was caused by the altered and/or reduced RAG-deficient thymocyte population. Expression of rearranged TCR transgenes in RAG-deficient mice is known to rescue the defect in mature T-cell production (9), and in this background a RAG-specific delay in tumorigenesis is not observed. Since it is unlikely that expression of the TCR transgenes also completely alters the mechanism of thymomagenesis, we conclude that inactivation of V(D)J recombination has no impact on the development of thymoma induced by p53 deficiency.

Our studies with *scid* mice also support the notion that V(D)J DSBs associated with an inability to rejoin coding ends do not promote thymoma. In the homozygous *scid* background, we observed no acceleration of thymoma with either TgTΔN or $p53^{-/-}$ mice. This result was surprising since previous reports showed that $p53^{-/-}$ *scid*/*scid* mice developed accelerated lymphoid malignancies compared to $p53^{-/-}$ mice (15, 28). In those studies, most mice (60 to 88%) developed predominantly B-cell lymphoma, although some T-cell lymphoma was observed. Thus, a high frequency of accelerated thymoma was not specifically observed. TgTΔN mice develop only thymoma; survival of TgTΔN mice homozygous at the *scid* locus is extended by 15%, and 62% of the mice develop thymoma. However, the difference in our study compared to previous reports was not specific to p53 inactivation by TΔN, since the $p53^{-/-}$ *scid*/*scid* mice in our study also showed no acceleration of death due to lymphoma. A plausible explanation for the difference between studies is that the *scid* mutation was in different genetic backgrounds—C.B-17 in previous studies and C57BL/6J here. Nacht et al. showed that the genetic background significantly affected the extent of lymphoma acceleration in $p53^{-/-}$ *scid*/*scid* mice relative to controls, with the effect being more dramatic in a 129/Sv-C57BL6-C.B-17 background than in a C57BL6-C.B-17 background (28). The $p53^{-/-}$ *scid*/*scid* mice generated in our study were entirely C57BL6, while the TgTΔN *scid*/*scid* mice were C57BL6-DBA. In addition to having the defect in V(D)J recombination, *scid* mice are defective in general DNA repair mechanisms (8, 43). In fact, the previous reports established that a DNA damage-inducible p53 checkpoint is intact in *scid* mice (7, 15, 28). Thus, it is possible that the acceleration of lymphoma previously observed reflects the absence of a general DNA damage checkpoint not specific to V(D)J recombination and that the level of damage, repair, or p53-independent checkpoint functions varies in distinct genetic backgrounds.

In this study, we also show that TCR translocations are not apparent in thymomas induced by a p53 deficiency. The chromosome painting analysis was performed blind, and the $ATM^{-/-}$ thymoma was readily characterized as harboring a TCR translocation. None of the other thymomas analyzed (all of which were induced by p53 deficiency) carried a TCR translocation detectable by this method. Chromosome painting is not sensitive enough to detect small deletions or insertions (34), and so we cannot rule out that aberrant rearrangement of TCR loci occurred at some level. However, this method readily detected a high frequency of TCR translocations in thymomas from $ATM^{-/-}$ mice (4). Thus, if TCR aberrations are involved in p53-deficient thymoma, the mechanism appears to be distinct from that caused by an ATM deficiency. Together with the genetic data described above, the chromosome analysis indicates that V(D)J recombination does not play a substantial role in thymomagenesis induced by p53 deficiency.

The observation that V(D)J recombination is not required for p53-deficient thymomagenesis is surprising since DNA damage-induced apoptosis of thymocytes has been considered the basis for tumor suppression in this cell type. Given that a high frequency of $p53^{-/-}$ mice and all TgTΔN mice spontane-

ously develop thymic lymphoma, thymocytes clearly need p53 tumor suppression for normal homeostasis. Perhaps T cells are susceptible to more general forms of DNA damage at some developmental stages. Alternatively, it is possible that the natural p53 signal does not involve DNA damage in these cells. Many aberrant conditions, including cell growth disruption, oxidative stress, and altered metabolic pools, induce p53 function in other cell types (see reference 22 for a review). The challenge now is to determine which, if any, of these conditions contributes to p53 tumor suppression in T cells. Although p53 is not required for apoptosis associated with clonal deletion of T cells (11, 23, 24), it could be required for apoptosis induced by an as yet unknown stimulus. Alternatively, p53 tumor suppression in thymocytes may involve a growth arrest checkpoint function in addition to, or instead of, apoptosis. For example, the consistent presence of aneuploidy in p53-deficient thymoma may indicate that loss of p53 checkpoint regulation leading to genetic instability has a key role in this tumor type.

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REFERENCES

1. **Abraham, K. M., S. D. Levin, J. D. Marth, K. A. Forbush, and R. M. Perlmutter.** 1991. Thymic tumorigenesis induced by overexpression of $p56^{lck}$. Proc. Natl. Acad. Sci. USA **88:**3977–3981.
2. **Adams, J. M., and S. Cory.** 1991. Transgenic models for haemopoietic malignancies. Biochim. Biophys. Acta Rev. Cancer **1072:**9–31.
3. **Aplan, P. D., et al.** 1997. An scl gene product lacking the transactivation domain induces bony abnormalities and cooperates with LMO1 to generate T-cell malignancies in transgenic mice. EMBO J. **16:**2408–2419.
4. **Barlow, C., S. Hirotsune, R. Paylor, M. Liyanage, M. Eckhaus, F. Collins, Y. Shiloh, J. N. Crawley, T. Ried, D. Tagle, and A. Wynshaw-Boris.** 1996. Atm-deficient mice: a paradigm of ataxia telangiectasia. Cell **86:**159–171.
5. **Bates, S., and K. H. Vousden.** 1996. p53 in signaling checkpoint arrest or apoptosis. Curr. Opin. Genet. Dev. **6:**12–19.
6. **Berns, A.** 1991. Tumorigenesis in transgenic mice: identification and characterization of synergizing oncogenes. J. Cell. Biochem. **47:**130–135.
7. **Bogue, M. A., C. Zhu, E. Aguilar-Cordova, L. A. Donehower, and D. B. Roth.** 1996. p53 is required for both radiation-induced differentiation and rescue of V(D)J rearrangement in scid mouse thymocytes. Genes Dev. **10:**553–565.
8. **Bosma, M., and A. Carroll.** 1991. The SCID mouse mutant: definition, characterization, and potential uses. Annu. Rev. Immunol. **9:**323–350.
9. **Chen, J., Y. Shinkai, F. Young, and F. Alt.** 1994. Probing immune functions in RAG-deficient mice. Curr. Biol. **6:**313–319.
10. **Chen, J., G. Tobin, J. M. Pipas, and T. A. Van Dyke.** 1992. T antigen mutant activities in transgenic mice: roles of p53 and pRB-binding in tumorigenesis of the choroid plexus. Oncogene **7:**1167–1175.
11. **Clarke, A. R., C. A. Purdie, D. J. Harrison, R. G. Morris, C. C. Bird, M. L. Hooper, and A. H. Wyllie.** 1993. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature **362:**849–852.
12. **Danska, J. S., and C. J. Guidos.** 1997. Essential and perilous: V(D)J recombination and DNA damage checkpoints in lymphocyte precursors. Semin. Immunol. **9:**199–206.
13. **Donehower, L. A., M. Harvey, B. L. Slagle, M. J. McArthur, C. A. J. Montgomery, J. S. Butel, and A. Bradley.** 1992. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature **356:**215–221.
14. **Froidevaux, S., and F. Loor.** 1991. A quick procedure for identifying doubly homozygous immunodeficient scid beige mice. J. Immunol. Methods **137:** 275–279.
15. **Guidos, C. J., C. J. Williams, I. Grandal, G. Knowles, M. T. F. Guang, and F. S. Danska.** 1996. V(D)J recombination activates a p53-dependent DNA damage checkpoint in *scid* lymphocyte precursors. Genes Dev. **10:**2038–2054.
16. **Hansen, R., and M. Oren.** 1997. p53; from inductive signal to cellular effect. Curr. Opin. Genet. Dev. **7:**46–51.
17. **Jacks, T., L. Remington, B. Williams, E. Schmitt, S. Halachmi, R. Bronson, and R. Weinberg.** 1994. Tumor spectrum analysis in p53-mutant mice. Curr. Biol. **4:**1–7.
18. **Kastan, M. B., O. Onyekwere, D. Sidransky, B. Vogelstein, and R. W. Craig.** 1991. Participation of p53 protein in the cellular response to DNA damage. Cancer Res. **51:**6304–6311.
19. **Kastan, M. B., Q. Zhan, W. S. El-Deiry, F. Carrier, T. Jacks, W. V. Walsh, B. S. Plunkett, B. Vogelstein, and A. J. J. Fornace.** 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell **71:**587–597.
20. **Kirsch, I. R., and F. Lista.** 1997. Lymphocyte-specific genomic instability and risk of lymphoid malignancy. Semin. Immunol. **9:**207–215.
21. **Larson, R. C., et al.** 1996. Protein dimerization between Lmo2 (Rbtn2) and Tal1 alters thymocyte development and potentiates T cell tumorigenesis in transgenic mice. EMBO J. **15:**1021–1027.
22. **Levine, A. J.** 1997. p53, the cellular gatekeeper for growth and division. Cell **88:**323–331.

22a. **Liao, M.-J., et al.** Unpublished data.

23. **Lowe, S. W., E. M. Schmitt, S. W. Smith, B. A. Osborne, and T. Jacks.** 1993. p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature **362:**847–849.
24. **McCarthy, S. A., H. S. Symonds, and T. Van Dyke.** 1994. Regulation of apoptosis in transgenic mice by SV40 T antigen-mediated inactivation of p53. Proc. Natl. Acad. Sci. USA **91:**3979–3983.
25. **Mombaerts, P., J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, and V. Papaioannou.** 1992. RAG-1-deficient mice have no mature B and T lymphocytes. Cell **68:**869–877.
26. **Morgan, S. E., and M. B. Kastan.** 1997. p53 and ATM: cell cycle, cell death, and cancer. Adv. Cancer Res. **71:**1–25.
27. **Nacht, M., and T. Jacks.** 1998. VDJ recombination is not required for the development of lymphoma in p53-deficient mice. Cell Growth Differ. **9:**131–138.
28. **Nacht, M., A. Strasser, Y. Chan, A. W. Harris, M. Schlissel, R. T. Bronson, and T. Jacks.** 1996. Mutations in the p53 and scid genes cooperate in tumorigenesis. Genes Dev. **10:**2055–2065.
29. **Nelson, W., and M. Kastan.** 1994. DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways. Mol. Cell. Biol. **14:**1815–1823.
30. **Nowell, P. C.** 1997. Genetic alterations in leukemias and lymphomas: impressive progress and continuing complexity. Cancer Genet. Cytogenet. **94:** 13–19.
31. **Oettinger, M.** 1992. Activation of V(D)J recombination by RAG1 and RAG2. Trends Genet. **8:**413–416.
32. **Pipas, J. M., K. W. Peden, and D. Nathans.** 1983. Mutational analysis of simian virus 40 T antigen: isolation and characterization of mutants with deletions in the T-antigen gene. Mol. Cell. Biol. **3:**203–213.
33. **Pircher, H., K. Burki, R. Lang, H. Hengartner, and R. M. Zinkernagel.** 1989. Tolerance induction in double specific T-cell receptor transgenic mice varies with antigen. Nature **342:**559–561.
34. **Qumsiyeh, M. B., and J. A. Peppers.** 1996. Sensitivity and specificity of whole chromosome paint (WCP) probes are correlated with size of translocated segment. Am. J. Med. Genet. **65:**173.
35. **Rabbitts, T. H.** 1994. Chromosomal translocations in human cancer. Nature **372:**143–149.
36. **Seabright, M.** 1971. A rapid banding technique for human chromosomes. Lancet **ii:**971–972.
37. **Shinkai, Y., G. Rathbun, K. Lam, E. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A. Stall, and F. Alt.** 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell **68:**855–867.
38. **Symonds, H. S., S. A. McCarthy, J. Chen, J. M. Pipas, and T. Van Dyke.** 1993. Use of transgenic mice reveals cell-specific transformation by a simian virus 40 T-antigen amino-terminal mutant. Mol. Cell. Biol. **13:**3255–3265.
39. **Taylor, A. M. R., J. A. Metcalfe, J. Thick, and Y.-F. Mak.** 1996. Leukemia and lymphoma in ataxia telangiectasia. Blood **87:**423–438.
40. **Van Dyke, T. A.** 1994. Analysis of viral-host protein interactions and tumorigenesis in transgenic mice. Semin. Cancer Biol. **5:**106.1–106.14.
41. **Verma, R. S., and A. Babu.** 1989. Human chromosomes: manual of basic techniques. Pergamon Press, New York, N.Y.
42. **von Boehmer, H.** 1990. Developmental biology of T cells in T cell-receptor transgenic mice. Annu. Rev. Immunol. **8:**531–556.
43. **Weaver, D. T.** 1995. What to do at an end: DNA double-strand-break repair. Trends Genet. **11:**388–392.
44. **Westphal, C. H., S. Rowan, C. Schmaltz, A. Elson, D. E. Fisher, and P. Leder.** 1997. Atm and p53 cooperate in apoptosis and suppression of tumorigenesis, but not in resistance to acute radiation toxicity. Nat. Genet. **16:**396–401.
45. **Xu, Y., T. Ashley, E. E. Brainerd, R. T. Bronson, M. S. Meyn, and D. Baltimore.** 1996. Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes Dev. **10:**2411–2422.