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Moloney Murine Leukemia Virus-Induced Lymphomas in p53-Deficient Mice: Overlapping Pathways in Tumor Development?

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The effect of Moloney murine leukemia virus (MoMLV) infection was examined in mice lacking a functional *p53* gene. Virus-infected $p53^{-/-}$ mice developed tumors significantly faster than uninfected $p53^{-/-}$ or virus-infected $p53^{+/+}$ littermates. However, the degree of synergy between MoMLV and the *p53* null genotype was weaker than the synergy between either of these and c-*myc* transgenes. A similar range of T-cell tumor phenotypes was represented in all *p53* genotype groups, including $p53^{-/-}$ mice, which developed thymic lymphomas as the most common of several neoplastic diseases. Lack of *p53* was associated with higher rates of metastasis and the ready establishment of tumors in tissue culture. Loss of the wild-type allele was a common feature of tumors in $p53^{+/-}$ mice and was complete in tumor cells in vitro, but this appeared to occur by a mechanism other than proviral insertion at the wild-type allele. A lower average MoMLV proviral copy number was observed in tumors of the *p53* null and heterozygote groups, suggesting that the absence of a functional *p53* gene reduced the number of steps required to complete the malignant phenotype. Mink cell focus-forming virus-like proviruses were detected in tumors of all infected mice but were relatively rare in *p53* null mice. Analysis of c-*myc*, *pim-1*, and *pal-1* showed that these loci were occupied by proviruses in some cases but at similar frequencies in *p53* wild-type and null mice. In conclusion, while inactivation of *p53* in the germ line predisposes mice to tumors similar in phenotype to those induced by MoMLV, it appears that virus-induced tumors generally occur without *p53* loss. We speculate that a *bcl-2*-like function carried or induced by MoMLV may underlie this *p53*-independent pathway.

Mutations in the *p53* gene are common in human tumors of many cell types (16). The role of *p53* as a tumor suppressor has been demonstrated unequivocally by the generation of transgenic mice lacking a functional *p53* gene. While these mice are viable and apparently normal, they are highly susceptible to the development of tumors, especially malignant lymphoma (6, 11, 30). *p53* has been described as the "guardian of the genome" (21), since its induction in response to DNA damage plays a critical role in growth arrest of cells in G_1 (19), while cells complete DNA repair or enter apoptosis if repair fails (42). In accord with this hypothesis, thymocytes from *p53* null mice fail to undergo apoptosis in response to radiation and other agents which damage DNA (8, 23).

The *p53* gene is frequently involved in Friend murine leukemia virus (MLV)-induced erythroleukemias, and inactivation of the gene by proviral insertion was an early clue to its role in suppressing tumor development (2, 26, 27). A recent study has shown that these *p53* mutations are acquired at a late stage in tumor development and are responsible for immortalization of the cell lines derived from murine erythroleukemias (17). However, inactivation of the *p53* gene by proviral insertion was first observed in an Abelson MLV-induced lymphoma (41) and has also been observed in a proportion of non-B-, non-T-cell lymphomas induced by Cas-Br-E virus (3). In contrast, there have been no reports of *p53* involvement in Moloney MLV (MoMLV)-induced thymic lymphomas.

To study the capacity of *p53* loss to act in synergy with MoMLV in thymic lymphomas, we tested the effect of viral infection in mice lacking a functional *p53* gene (11). These studies demonstrate that viral infection can accelerate tumorigenesis in *p53* null mice and provide evidence that the number of steps required for full malignant transformation is reduced in *p53* null tumors. However, the relative weakness of the interaction between MoMLV infection and *p53* loss suggests that there is an overlap in their oncogenic effects.

MATERIALS AND METHODS

Transgenic mice. The generation of an inactive *p53* gene and its introduction into the mouse germ line were described previously (11). The breeding stock used for these experiments were *p53* heterozygote mice which had been crossed with NIH mice (20). The *p53* heterozygote offspring from the breeding stock were bred together to generate the experimental groups of sibling mice with homozygous null, heterozygote, and *p53* wild-type genotypes, which were then infected on neonatal day 1 with 10^5 infectious units of MoMLV (39).

DNA hybridization analysis. High-molecular-weight DNA was prepared from mouse tissues by using guanidinium chloride. DNA digestion with restriction enzymes, separation by agarose gel electrophoresis, hybridization, and washing of blots were all carried out as described previously (28). Transfer to Hybond N membranes was carried out as recommended by the manufacturer (Amersham International plc.).

Probes were radiolabelled by nick translation or random priming, using [α-^{32}P]dCTP (>3,000Ci/mmol; Amersham) to specific activities of greater than 10^8 cpm/μg of DNA.

The *p53* null allele was detected by Southern blotting using a PCR-generated *p53* exon 4 probe (primers 5′-CCATCACCTCACTGCATG-3′ and 5′-GTGCACATAACAGACTTGGC-3′) which also revealed wild-type and pseudogene alleles of *p53*.

Tumors were screened for rearrangements in the c-*myc* and N-*myc* genes with probes generated by PCR from mouse genomic DNA, using published sequences (4, 36). The c-*myc* sequence was amplified by using the primers 5′-CAAGGAAGGACTATCCAG-3′ and 5′-CCTCCTCGAGTTAGGTCA-3′, while the N-*myc*

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TABLE 1. Tumor incidence and phenotype in *p53*-deficient, MoMLV-infected mice

p53 genotype	MoMLV infection	*n*	No. of:			Age range (days)
			Thymic lymphomas	Disseminated lymphomas	Other tumor types[a]	
−/−	−	18	12	1	12	96–231
−/−	+	14	13	1	4	71–136
+/−	+	24	16	5	4	99–267
+/+	+	11	11	0	0	126–360

[a] Include hemangiosarcoma, osteosarcoma, fibrosarcoma, adenocarcinoma, and teratoma.

primers were 5′-TCACTCCTAATCCGGTCA-3′ and 5′-TGAGTCGCTCAAG GTAT-3′.

Tumors were screened for rearrangements in the *pim-1* locus by using a probe previously described (clone A [9]) and in the *bmi-1* and *pal-1* loci by using the mouse *bmi-1* cDNA clone 13.1 and the *pal-1* probe 11A2 (39). The tumors were also screened for rearrangements in the *ahi-1* locus as described previously (29).

Rearrangements of the T-cell receptor (TCR) β-chain gene and the immunoglobulin heavy-chain (IgH) gene were determined by using a 496-bp PCR-generated fragment of the Cβ region, derived from the 1.2-kb fragment of clone 86T5 (15), and a 1.7-kb *Eco*RI-*Bam*HI fragment of plasmid J11 (24), respectively.

MoMLV proviral sequences were detected by using a probe derived from the U3 domain of the long terminal repeat (10) with extended (30-min) washes at high stringency (0.1× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]).

Flow cytometry. Cells were prepared for flow cytometry by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation at 3,000 × *g* for 10 min followed by resuspension in phosphate-buffered saline containing 0.1% sodium azide at 10^6 cells per ml. Cells were directly labelled for 30 min at 4°C, using the following antibodies: R-phycoerythrin-conjugated rat anti-mouse CD4, fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD8 (both from Life Technologies), FITC-conjugated B220 (AMS Biotechnology), Quantum red-conjugated rat anti-mouse CD3 (Sigma Immunochemicals), and FITC-conjugated hamster anti-mouse αβ TCR (Pharmingen).

RESULTS

Interaction of *p53* loss and MoMLV infection in thymic lymphoma. Mice homozygous for an inactivated *p53* gene (11) and their heterozygous or wild-type littermates were infected at 1 day of age with 10^5 U of MoMLV clone 1A (39). The infected mice and noninfected controls of the same genetic background were monitored for tumor development over a period of 9 months. The patterns of tumor formation in these mice are summarized in Table 1 and Fig. 1.

As indicated in Table 1, lymphoma of thymic origin was the predominant tumor type in all groups, although $p53^{-/-}$ mice developed a range of other malignant diseases, some mice presenting with more than one primary neoplasm. The kinetics of thymic lymphoma development in the infected mice, sorted according to *p53* genotype, is shown in Fig. 1A. The rate of tumor onset in infected $p53^{-/-}$ mice was significantly different from that in infected $p53^{+/+}$ mice ($P < 0.001$, Mann-Whitney rank sum test). The rate of tumor onset in infected $p53^{-/+}$ mice was also significantly different from that in infected wild-type mice ($P < 0.05$). Further evidence of an additive interaction between MoMLV infection and *p53* loss is shown in Fig. 1B: the rate of thymic lymphoma onset in virus-infected $p53^{-/-}$ mice was significantly different from that in noninfected controls ($P < 0.001$).

The surface phenotypes of thymic lymphomas in MoMLV-infected mice are similarly heterogeneous, regardless of *p53* genotype. Flow cytometry was used to compare the expression of lymphoid cell surface markers on the tumor cells from the various genotypes. There were no significant differences in the frequency of tumors of any phenotype in either infected *p53* null, infected heterozygous, or wild-type-infected mice. The thymic lymphomas appeared to be exclusively of T-cell origin: all tumors stained positive for CD3, CD4, CD8, or TCR β chain (including three which appeared positive for CD45R [B220], which is also expressed on pre-B cells). The results are in accord with previous phenotypic analyses of MoMLV-induced lymphomas which were found to be heterogeneous and representative of early to intermediate stages of T-cell development (7, 22). Analysis of eight thymic lymphomas from uninfected *p53* null mice revealed a similarly broad range of T-cell phenotypes.

Analysis of proviral integrations shows lower proviral copy numbers in *p53* null lymphomas. Since *p53* null mice developed T-cell lymphomas similar in phenotype to those induced by MoMLV, it was conceivable that some of the tumors arising in the MoMLV-infected *p53* null mice occurred in uninfected cells or in cells infected after malignant clonal expansion. Such tumors would be expected to show, respectively, no evidence of viral integration or a polyclonal pattern of virus-host junction fragments. We were also interested in the possibility that *p53* null lymphomas require fewer mutagenic events to complete the malignant transformation process. If so, *p53* null tumors might be expected to have undergone fewer rounds of proviral integration and show a lower copy number.

Analysis of integrated proviruses by hybridization with a U3 probe showed that all of the lymphomas from MoMLV-infected mice contained clonal integrations, regardless of their *p53* genotype. The number of U3-hybridizing proviral junction

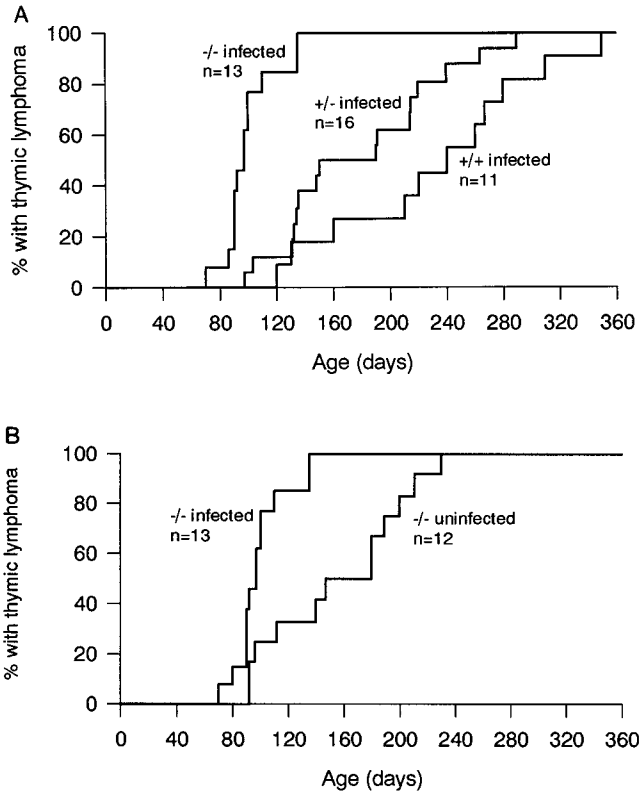

FIG. 1. (A) Onset of thymic lymphoma in MoMLV-infected, *p53* mutant mice. Tumor onset was significantly faster in null than in wild-type mice ($P < 0.001$) and also in heterozygous than in wild-type mice ($P < 0.05$). (B) MoMLV infection and onset of thymic lymphoma in *p53* null mice. The onset is significantly faster in infected null mice than in uninfected mice of the same genetic background ($P < 0.001$).

TABLE 2. Proviral copy number in tumors of *p53*-deficient, MoMLV-infected mice

Genotype	*n*[a]	Proviral copy no./ no. of cell clones
Null	9	4.2[b]
Heterozygote	7	4.4[b]
Wild type	8	10.1[b]

[a] For some tumors, DNA samples were not adequate for Southern blot analysis.

[b] Sample sets show significant differences: $P < 0.001$ (null/wild type) and $P < 0.004$ (heterozygote/wild type) (Mann-Whitney rank sum test).

fragments was estimated for each tumor. This value was corrected for the number of clones detected by TCR or immunoglobulin gene rearrangement to derive an estimated copy number per cell clone, as shown in Table 2. These results showed that there was a significantly higher proviral copy number in the tumors of $p53^{+/+}$ mice than in those of their $p53^{-/-}$ and $p53^{+/-}$ siblings.

Gene rearrangements at the TCR Cβ1 and IgH loci were examined and confirmed the monoclonal or oligoclonal nature of the tumors. The clonal complexity of tumors from *p53*-deficient mice was not significantly different from that in the wild-type-infected controls.

MCF virus-like proviruses are relatively rare in tumors of *p53*-deficient mice. MoMLV-induced lymphomas frequently show the presence of recombinant mink cell focus-forming (MCF) viruses. Direct enumeration of MCF virus recombinants by hybridization analysis is complicated by the presence of endogenous proviruses with related *env* sequences. However, a convenient surrogate marker for the presence of such recombinants is a conserved *Eco*RI site in a domain of the *env* gene which is consistently derived from the endogenous virus parent in the recombination process (38). The presence of U3-hybridizing *Eco*RI fragments shorter than MoMLV genome length serves as an indication of the presence of MCF virus recombinants. Analysis of the tumors by this criterion showed that the $p53^{-/-}$ tumors contained very few short *Eco*RI fragments detected by the U3 probe, while increasing proportions of such fragments were seen in the $p53^{+/-}$ and $p53^{+/+}$ lymphomas (Fig. 2 and Table 3).

Gene rearrangements in MoMLV-induced lymphomas: c-*myc* and *pim-1* are frequent targets for proviral insertion in tumors differing in *p53* status. We were interested in the possibility that *p53* loss was associated with the activation of a preferred set of *p53*-collaborating genes. Alternatively, some gene activation events mediated by proviral insertion might be rendered redundant in the absence of *p53* and would therefore be lacking from the *p53* null tumor group.

Tumors were screened for insertions in several integration sites, i.e., c-*myc*, *pim-1*, *bmi-1*, *pal-1*, *ahi-1*, and N-*myc*, with restriction digests selected to span the major loci of proviral insertion (9, 14, 39). No insertions were seen at the N-*myc*, *ahi-1*, and *bmi-1* loci.

Of 27 tumors screened, 9 had rearrangements of the c-*myc* gene. These were shown to be tumor specific by comparison with control tissue (tail) DNA, and rescreening of blots with a U3 probe indicated that they were due to proviral insertions. Analysis of *Kpn*I digests showed that all of the proviral insertions were in the opposite orientation to the direction of transcription of c-*myc*. As reported previously, the size of the rearranged *Eco*RI fragment was consistent with the presence of an integrated MCF provirus in some cases (33). The rearrangements at *pim-1* and *pal-1* were also shown to be due to proviral insertion but were not mapped in detail.

The data are summarized in Table 4. All of the rearrangements in the *p53* null tumors occurred individually, but one wild-type tumor showed both *pim-1* rearrangement and *p53* allele loss.

Frequent *p53* wild-type allele loss from heterozygote and wild-type tumors: no evidence for insertional mutagenesis. The structure of the *p53* locus was determined for six of the *p53* heterozygote tumors. In all six of these tumors, the intensity of the wild-type *p53* allele was greatly reduced. No rearranged fragment was detected, suggesting that the wild-type allele had been lost by a nonviral mechanism such as chromosomal nondisjunction. In one tumor (*p53* 71i), no trace of the wild-type allele could be seen (data not shown).

One of the eight wild-type tumors screened showed a rearrangement at *p53*. The rearrangement was not seen in the overlapping *Bgl*II fragment and was probably the result of a mutation which created a novel *Eco*RI site. Screening of a larger number of MoMLV-induced lymphomas confirmed this as an uncommon event in MoMLV-induced lymphomas.

Cell lines were established from all *p53* null tumors and also from three tumors from *p53* heterozygous mice. The cell lines from heterozygous tumors showed complete loss of the wild-type allele. This result is in accord with studies which showed the ready establishment of cell lines from tissues of *p53* null mice (37), and it would appear that a strong growth advantage exists for *p53* null cells in vitro.

Clonal evolution in MoMLV-induced, *p53* heterozygote tumors: accumulation of genetic changes. Extensive metastases were seen in both infected *p53* null and infected heterozygous mice. This result, together with the loss of the wild-type allele from metastatic cells in heterozygous mice, suggests that loss of *p53* function can contribute to metastatic potential in MoMLV-induced tumors.

The evolution of two lymphomas of $p53^{+/-}$ mice is shown in detail in Fig. 3, in which thymic tumor DNA is compared with kidney DNA. The latter tissue frequently showed evidence of metastatic involvement. Analysis of TCR β-chain and IgH rearrangements (Fig. 3a and b) indicated the clonal or oligo-

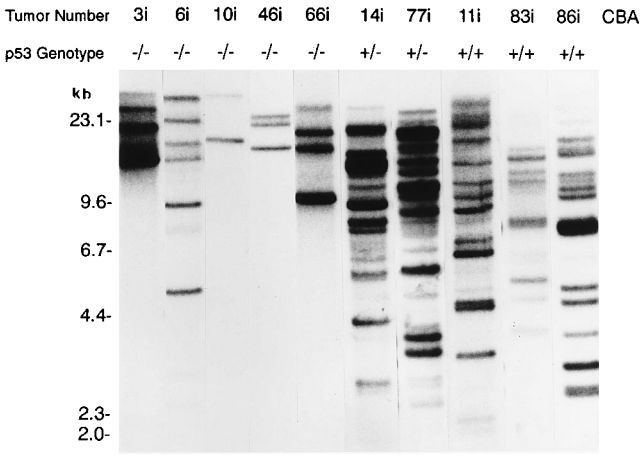

FIG. 2. Proviral integrations in tumors of *p53*-deficient, MoMLV-infected mice. Southern blots of *Eco*RI-digested DNAs were hybridized with a U3 probe which showed that *p53* null (−/−) and heterozygote (+/−) tumors had lower numbers of integrations than *p53* wild-type (+/+) tumors. Hybridizing fragments of less than 8.8 kb suggested the presence of defective viruses or recombinant MCF viruses, since the MoMLV genome lacks an *Eco*RI site. These were least abundant in the *p53* null tumors (see Table 3).

TABLE 3. Estimation of MCF virus or defective proviral content of tumors by *Eco*RI analysis

p53 genotype	*n*	No. of *Eco*RI fragments >8.8 kb in each tumor	Mean ± SD	No. of *Eco*RI fragments <8.8 kb in each tumor[a]	Mean ± SD
−/−	8	3, 4, 2, 3, 3, 3, 5, 1	3.0 ± 1.2	0, 6, 0, 2, 0, 0, 1, 1	1.3 ± 2.1
+/−	4	6, 4, 3, 10	5.8 ± 3.1	8, 4, 2, 9	5.8 ± 3.3
+/+	7	6, 8, 2, 7, 5, 4, 7	5.6 ± 2.1	8,10, 9, 10, 8, 5, 9	8.4 ± 1.7

[a] Significant differences were observed between the numbers of fragments <8.8 kb in the *p53* null and wild-type tumors ($P < 0.001$; *t* test) and between *p53* null and heterozygote tumors ($P < 0.02$; *t* test). An *Eco*RI fragment of less than 8.8 kb suggests integration of MCF-like provirus.

clonal nature of the tumors in each case. For tumor 14i, it appeared that a predominant clone with a unique TCR Cβ1 rearrangement (14-kb *Hin*dIII fragment) was present in both the thymic tumor and kidney metastases. In thymus, loss of the unrearranged Cβ1 alleles (9.5 kb) was almost complete, confirming gross pathology which suggested that this tissue was almost entirely replaced by tumor. However, only the thymic tumor showed evidence of IgH rearrangement, suggesting that this was a later event which occurred only in the thymic tumor cell population. Tumor 77i appeared to be more complex by TCRβ rearrangement, and the kidney metastases contained discrete subpopulations of matching genotype. In both tumors, depletion of the wild-type *p53* allele had occurred (Fig. 3c), as shown by the reduced intensity of the 5.5-kb *Bam*HI wild-type band relative to the 7.5-kb null allele. Since this depletion was also evident in kidney DNA, in which residual normal tissue was also present, it appeared that the metastases were largely derived from *p53* null cells. Both tumors showed evidence of proviral insertion at the c-*myc* locus, as indicated by the presence of a 7.5-kb *Kpn*I fragment rearranged allele in addition to the 10-kb germ line species. The greater intensity of the rearranged c-*myc* allele in tumor 14i indicated that gene amplification had also taken place, while the high representation of the rearranged species in both kidney DNAs was indicative of selection for cells with amplifications of the abnormal allele in metastases. Finally, both tumors showed evidence of *pim-1* rearrangements, which were detected as 10-kb *Eco*RV fragments compared with the 18-kb germ line species. Again, there was evidence of enrichment of cells carrying the rearrangement in metastatic deposits.

DISCUSSION

The acceleration of tumor onset in MoMLV-infected *p53* null mice, combined with the loss of the wild-type allele in tumors of heterozygous mice, shows that loss of *p53* function can contribute to the development of MoMLV-induced thymic lymphoma. Although *p53*-deficient mice developed thymic lymphomas of a similarly broad range of phenotypes in the absence of MoMLV, all tumors in virus infected mice contained unique proviral integrations, indicating that integration preceded clonal outgrowth of the tumor cell. However, despite this clear evidence of an interaction between MoMLV infection and the *p53* null genotype, the degree of synergy that we observed here was weak compared with the very potent effect of MoMLV infection in mice carrying c-*myc* transgenes (34, 39) and the similarly strong interaction between the *p53* null genotype and a c-*myc* transgene directed to the T-cell compartment (6).

A possible explanation for these observations is that a functional overlap exists between MoMLV infection and loss of *p53* in T-cell lymphomagenesis. A similar explanation was invoked recently to account for the lack of synergy between the *p53* null genotype and a *bcl-2* transgene (25). Furthermore, a similar lack of synergy was noted between the same *bcl-2* transgene and MoMLV infection, since the rate of tumor onset was the same as in nontransgenic controls (1). Again, the *bcl-2* transgene is strongly synergistic with c-*myc* (35), and this synergy has been attributed to the capacity of *bcl-2* to interfere with apoptosis induced by overexpressed Myc (5). Taking these observations together, we postulate that the functional overlap between MoMLV, *p53* loss, and *bcl-2* activation is due to their antiapoptotic effects. However, the effects are likely to be context and cell type specific. This hypothesis could also account for the apparent divergence in tumorigenic mechanism by the prototypic MLVs, MoMLV and Friend MLV. If the equivalent antiapoptotic viral function is lacking in Friend MLV-induced erythroleukemias, direct inactivation by viral insertional mutagenesis (2, 26, 27) would be favored.

The postulated overlap in function of MoMLV and *p53* loss is clearly not complete, as indicated by the accelerated onset and unique properties of tumors in *p53* null, MoMLV-infected mice. The unique functional contribution of *p53* loss was indicated by the high metastatic potential of such tumors and their ready establishment in tissue culture. Moreover, the activation of c-*myc* by MoMLV in such tumors demonstrates that the virus can activate a class of genes which act in synergy with *p53* loss (6, 12, 18).

In view of the frequency with which Friend MLV insertion inactivates *p53*, it was somewhat surprising that we did not detect this phenomenon even in tumors of *p53* heterozygotes, in which knockout of the remaining functional allele would have been expected to occur quite readily. Loss of the normal allele did occur in most heterozygote tumors, but there was no evidence of novel rearrangements suggestive of proviral insertion at the gene. Since this appeared to be a late event in tumor development, it is possible that at this late stage, interference to superinfection rendered insertional inactivation of *p53* less likely than other mechanisms such as chromosomal nondisjunction.

The accelerated tumor onset and reduced average proviral copy number in tumors of *p53*-deficient mice suggests that fewer steps may be required to establish the fully malignant phenotype in such cases. We were therefore interested in looking for evidence of specific gene activation events which would be preferred in the presence of *p53* loss or gene activation events which could substitute for *p53* loss and would therefore

TABLE 4. Proviral integration sites and *p53* allele loss in *p53*-deficient, MoMLV-infected mice

p53 genotype	No. of mice with insertion or loss/no. examined			
	c-*myc* insertions	*pim-1* insertions	*pal-1* insertions	p53 allele loss
−/−	3/11	0/11	1/11	NA
+/−	4/8	2/8	0/8	6/8
+/+	2/8	2/8	0/8	1/8

[a] NA, not applicable.

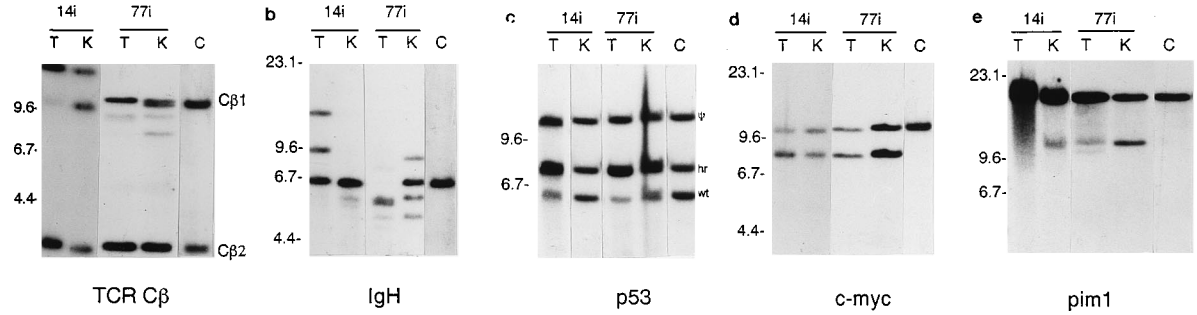

FIG. 3. Analysis of tumor progression in *p53* heterozygote mice 14i and 77i. Thymic tumor (lanes T) and corresponding kidney (lanes K) DNA samples, as well as controls (lanes C), were analyzed by Southern blot hybridization. (a) Analysis of *Hin*dIII-digested DNA with a TCR Cβ probe. The Cβ1 and Cβ2 germ line fragments are 9.5 and 3 kb, respectively. (b) Analysis of *Eco*RI digests with an IgH probe. The germ line fragment is the 6.6-kb species. (c) *Bam*HI digests analyzed with a *p53* gene probe, detecting the wild-type (5.5 kb) and inactivated (7.5 kb) alleles and the pseudogene (11 kb). (d) *Kpn*I digests analyzed with a c-*myc* probe, showing the unrearranged allele (10 kb) and rearrangements consistent with proviral insertions 5′ to the locus (7.5 kb). (e) *Eco*RV digests analyzed with a *pim-1* probe. The unrearranged allele is represented by the 18-kb fragment, while rearrangements consistent with proviral insertion appeared at around 8 to 10 kb. Sizes are indicated in kilobases.

be absent from the tumors in which *p53* was inactive. Insertions were detected at three loci (c-*myc*, *pim-1*, and *pal-1*), but none of these appeared to be preferentially represented in the *p53* null or wild-type groups. While insertions at *pim-1* were not seen in the *p53* null group, they were present in *p53* heterozygote tumors which displayed wild-type allele loss.

It was interesting that proviral insertions at both c-*myc* and *pim-1* in *p53* heterozygote mice were associated with loss of the wild-type allele, suggesting that all three lesions may have collaborated in the genesis of these tumors. The implied existence of at least three gene complementation groups in thymic lymphosarcoma is in accord with previous studies which showed that *pim-1* can collaborate with either c-*myc* or *bcl-2* in lymphoma development (1, 40). However, we found no evidence for a set of gene activation events which replaces or is specifically favored by *p53* loss. Continued screening of proviral insertion sites in $p53^{+/+}$ and $p53^{-/-}$ lymphomas might reveal such a gene set if it exists.

We noted that MCF virus-like elements were generated in many of the tumors examined here but were significantly underrepresented in lymphomas of $p53^{-/-}$ mice. This does not simply reflect the lower number of proviral integrations in *p53* null tumors, but it is possible that this observation is due to the slower onset of tumors in $p53^{+/+}$ mice and hence the longer time available for MCF virus recombinants to arise. However, it is also possible that a direct role was played by MCF viruses. First, MCF viruses enter murine cells via a different receptor and would therefore overcome interference barriers to tumor progression by sequential proviral integration events (31). Second, a recent study has shown that MCF virus and xenotropic MLV induce interleukin-9 (IL-9) expression in rat T cells and promote IL-2-independent growth by concomitant insertion at the IL-9 receptor (13). Since IL-9 is a potent antiapoptotic factor for murine lymphoma cells (32), this observation provides a potential rationale for the apparent bypass of *p53* loss in most MoMLV-induced thymic lymphomas. However, whether MoMLV itself mediates a similar effect or must operate via MCF virus recombinants requires further investigation.

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