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Infrequent *p53* Mutation in Mouse Tumors with Deregulated *myc*

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Abstract

An invariant genetic lesion in mouse plasmacytomas is deregulated expression of c-*myc* as a consequence of chromosomal translocation. However, retroviral and transgenic studies suggest that additional genetic lesions may contribute to the genesis of plasmacytomas. The *p53* tumor suppressor gene is a likely contributor to this genetic lesion, since there is a high incidence of *p53* mutation in Burkitt's lymphomas and B-ALL (L3), both of which contain translocations involving c-*myc* analogous to those in plasmacytomas. In addition, *p53* has been shown to be a transcriptional modulator of c-*myc* expression. In a survey of 27 mouse plasmacytomas by single-strand conformation polymorphism, we identified a single mutation (3.7% incidence), suggesting that *p53* lesions are not frequent contributors to plasmacytomagenesis. A similar study of macrophage-monocyte tumors generated by a c-*myc*-containing retrovirus also indicates a lack of *p53* involvement in deregulated c-*myc* expression. These results suggest that the specific maturation stage of transformed B-lymphocytes, independent of c-*myc* deregulation, may be the critical factor which determines the involvement of mutant *p53*.

Introduction

Mouse plasmacytomas characteristically display a nonrandom reciprocal chromosomal translocation involving mouse chromosome 15 (for reviews, see Refs. 1 and 2). The chromosomal translocations occur in or near the c-*myc* gene or in a second cluster 260 kilobases downstream in a region called Pvt-1, and, in every case, this translocation results in deregulation of c-*myc* gene expression. Several lines of evidence suggest that deregulated c-*myc* is not the sole lesion in the genesis of mouse plasma cell tumors (1, 2). In the search for additional genetic lesions, it seems reasonable to begin with genes that are known to interact with c-*myc* in a cooperative manner. For example, it is known that *ras* and *myc* can cooperate in the neoplastic transformation of primary mouse fibroblasts (3). Thus, *ras* gene mutation could be considered a good candidate gene for such a secondary lesion. However, a survey of mouse plasmacytomas has revealed no significant incidence of *ras* mutation.[2]

Studies of mouse plasmacytomas have revealed that among tumors with near-diploid karyotypes, trisomy 11 was frequently observed in addition to the requisite t(12;15) or t(6;15) chromosomal translocation involving the *myc*/Pvt-1 loci of chromosome 15 (4). This finding prompted an investigation of the tumor suppressor gene *p53*, which is found on chromosome 11 in the mouse. Somatic point mutations in the *p53* gene have been found in a vast array of tumor types including Burkitt's lymphoma and B-ALL (L3), which exhibit deregulation of the c-*myc* gene very similar to that found in most mouse plasmacytomas (5–7). However, in most other human lymphomas in which c-*myc* deregulation has not been established as the primary pathogenetic lesion, *p53* mutations are infrequent (5). The expression of *p53* has also been shown to be modulated by helix-loop-helix-containing transcription factors such as c-*myc* (8). Therefore, we reasoned that mutated *p53* might be an excellent candidate for a genetic lesion contributing to plasmacytomagenesis. Since a majority of mutations in *p53* have been found to be clustered to the regions of exons 5–8, we concentrated on this area of the gene for our analysis (9, 10).

We utilized SSCP[3] and direct sequencing of PCR-amplified DNA to survey pristane-induced plasmacytomas for *p53* mutation. Among the tumors included in this experiment are several that have been previously characterized with trisomy of chromosome 11 (4). Finally, tumors which contain deregulated *myc* introduced in the form of a retroviral construct rather than occurring vis-à-vis chromosomal translocation were also examined.

Materials and Methods

Mouse plasmacytomas were induced in adult BALB/c by three regimens: (*a*) i.p. injection of pristane (2,6,10,14-tetramethylpentadecane), referred to as TEPC, FLOPC or SP2/0; (*b*) i.p. pristane inoculated with Abelson virus (A-MuLV), referred to as ABPCs; or (*c*) i.p. pristane inoculated with a retrovirus expressing v-*raf*/v-*myc*, referred to as J3-PCs (1). Monocyte-macrophage (McML) tumors were generated by i.p. inoculation of a c-*myc*/*neo*/M-MuLV retroviral construct in combination with pristane treatment (11).

A total of 27 plasmacytomas were analyzed for *p53* mutations, 16 of which had been induced by pristane alone (TEPC, FLOPC, SP2/0) and contained deregulated endogenous c-*myc* (12).[4] In eight plasmacytomas induced with pristane plus Abelson virus (ABPC) endogenous c-*myc* is also deregulated. These eight tumors had previously been karyotyped with trisomy 11 (4). The remaining three plasmacytomas (J3PC) contained a retrovirus that overexpressed v-*myc*/v-*raf*, and, therefore, the endogenous c-*myc* remains translocation-negative (13). Three macrophage-monocytic (McML) tumors were also translocation-negative as a result of induction with pristane plus a retrovirus containing deregulated c-*myc* (11).

Oligonucleotides required for sequencing and for the PCR amplification in SSCP analysis were synthesized on an ABI DNA synthesizer. Sequences of the oligonucleotide primers were obtained from published sequences (14) and include the following: exon 5, TACTCTCCTCCCCTCAATAAG (sense), ACCATCGGAGCAGCCCTCAT (antisense); exon 6 CTGGCTCCTCCCCAGCATCTT (sense), CTCGGGTGGCTCATAAGGTAC (antisense); exon 7, GCCGGCTCTGAGTATACCACC (sense), CTGGAGTCTTCCAGTGTGATG (anti-sense); exon 8, GGGAACCTTCTGGGACGGGAC (sense), TCTCTTTGCGTCCCCTGGGGG (antisense). SSCP analysis was performed as described by Orita *et al.* (15) with a few modifications. Initial PCR was performed with 250 ng of DNA and 20 pmol of each flanking primer. The reaction mixture contained 5 μM of deoxynucleo-

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[2] K. Huppi, unpublished data.

[3] The abbreviations used are: SSCP, single-strand conformation polymorphism; PCR, polymerase chain reaction.

[4] K. Huppi and J. F. Mushinski, unpublished observations.

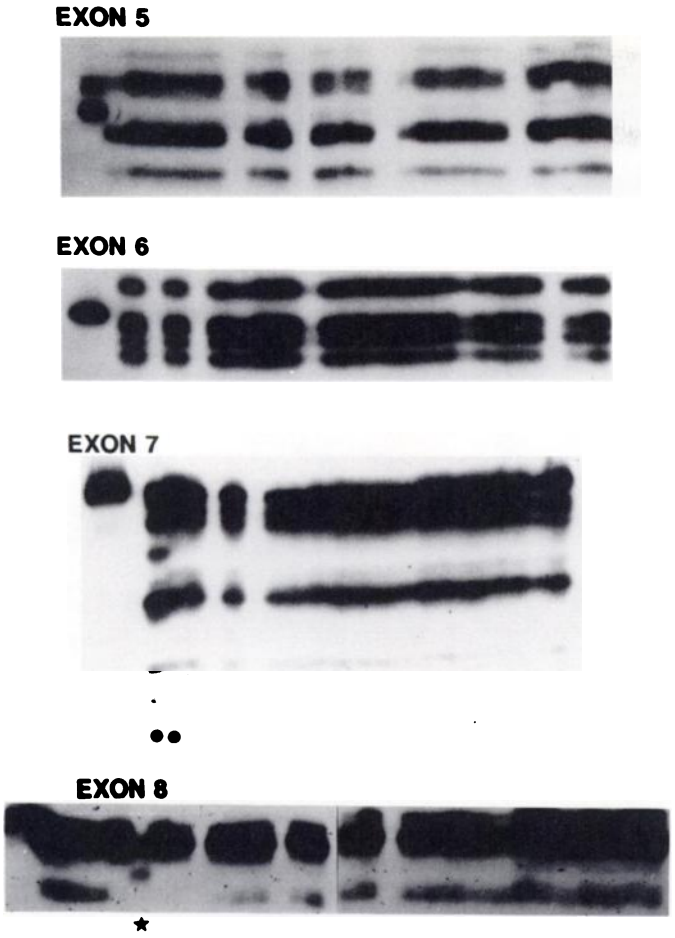

Fig. 1. SSCP analysis of *p53* mutations in mouse tumors. PCR-amplified genomic DNA fragments generated from exons 5, 6, 7, and 8 of mouse *p53* were electrophoresed on 6% polyacrylamide gels at room temperature. *Left*, nondenatured (double-stranded) fragments for each exon. Positive controls corresponding to MethA and S49 are denoted by ● in the exon 7 panel. The plasmacytoma J3PC2139 carries a mutation in exon 8 of *p53* and is marked by a ★.

tide triphosphates with 10 mM Tris (pH 8.5), 50 mM KCl, and 1 mM $MgCl_2$. Approximately 2 μCi of [α-^{32}P]dCTP was added to each reaction mixture prior to initiating PCR amplification by adding 1 unit of Taq polymerase. Thirty cycles of denaturation (94°C), annealing (58°C, exons 5 and 8), (62°C, exons 6 and 7), and extension (72°C) were done in a DNA thermal cycler (Perkin-Elmer). Samples were denatured at 95°C for 5 min, loaded onto 6% polyacrylamide (19:1 acrylamide:bisacrylamide ratio) gels and electrophoresed overnight at room temperature. A methylcholanthrene-induced sarcoma with *p53* mutations in exons 5 and 7 (16) and a T-cell lymphoma with a *p53* mutation[5] in exon 7 were included as positive controls (Fig. 1). Double-stranded (nondenatured) DNA products of each PCR reaction were included in a lane of the SSCP electrophoresis to permit identification of the PCR products.

Since the mouse also contains a pseudogene in addition to the structural *p53* gene (17), theoretically exons from both genes could be amplified to a similar extent. We suspect that the pseudogene of *p53* in reality represents only a minor product of the PCR amplification reaction, because the expressed *p53* sequence of each of the primer sets for exons 5, 6, 7, and 8 contains at least one base difference from the pseudogene. Thus, PCR amplification of the pseudogene of *p53* would be much less efficient than the expressed gene.

For sequencing, PCR products were denatured at 98°C for 2 min, and the appropriate primers were annealed at room temperature. The labeling and extension were carried out at room temperature using the Sequenase reaction kit (USB).

[5] K. Huppi, unpublished data.

Results and Discussion

Disruption of regulated c-*myc* expression can be achieved by chromosomal translocation, somatic point mutation, or retroviral integration within a region spanning approximately 260 kilobases of the *myc*/Pvt-1 locus on mouse chromosome 15. Consequently, deregulation of c-*myc* is more readily ascertained by Northern analysis of endogenous c-*myc* RNA levels. By this criterion, nearly 100% of mouse plasmacytomas have been determined to exhibit deregulated c-*myc* expression (12). Similarly, we have assayed *p53* RNA levels in mouse plasmacytomas and have found no hint of correlation between expression of *p53* RNA and tumorigenicity (data not shown). Furthermore, we do not find any increase in *p53* RNA levels, particularly in plasmacytomas with trisomy 11. Thus, it appears that any involvement of *p53* in plasmacytomagenesis must be found at the DNA level in the form of somatic point mutations.

To determine the frequency of *p53* mutation in mouse tumors, we chose a representative panel of lymphoid or monocytic tumors containing deregulated c-*myc* gene expression. We observed an SSCP polymorphism in only 1 of 30 tumors (Fig. 1; Table 1). Analysis of mutation in the expressed *p53* gene could be complicated by the mouse *p53* pseudogene in that it is possible that some of the minor SSCP bands in Fig. 1 could represent *p53* pseudogene sequences (17). Nonetheless, variants were not observed among those minor SSCP bands, either. Possible mutations residing within the primer sequences themselves will also be missed by this assay but still represent only a small proportion of the total *p53* target sequence.

The single mutation was identified in *p53* exon 8 of the J3 tumor, J3PC2139, which was induced by pristane in combination with a *raf*/*myc* retroviral construct (13). To verify the exact

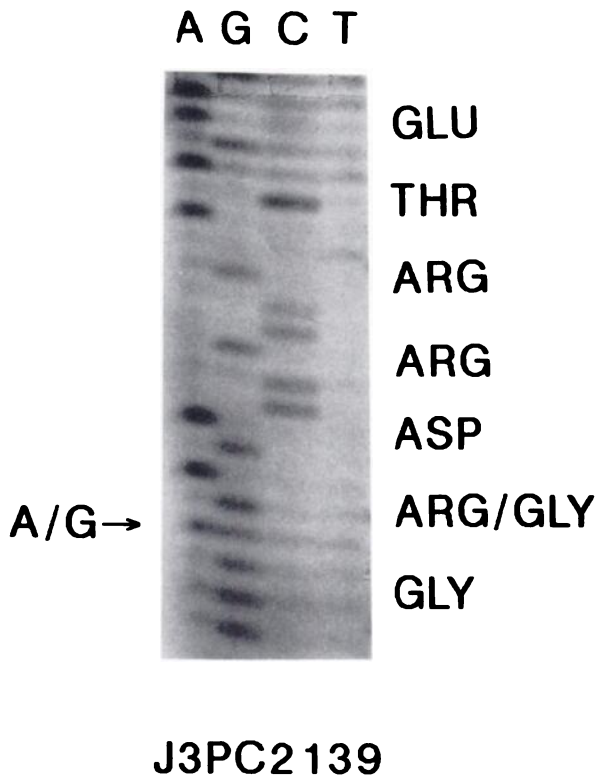

Fig. 2. Sequence of *p53* exon 8 mutation in J3PC2139. PCR-amplified DNA from J3PC2139 was sequenced by the double-stranded method. The presence of both A and G nucleotides indicates that both mutant and wild-type forms are found.

Table 1 *Mouse tumor survey*

Tumor	Induction	p53 mutations				Trisomy 11
		Exon 5	Exon 6	Exon 7	Exon 8	
Plasma cell tumors						
ABPC22	A-MuLV; prist.[a]	−	−	−	−	+
ABPC26	A-MuLV; prist.	−	−	−	−	+
ABPC33	A-MuLV; prist.	−	−	−	−	+
ABPC48	A-MuLV; prist.	−	−	−	−	−
ABPC65	A-MuLV; prist.	−	−	−	−	+
ABPC89	A-MuLV; prist.	−	−	−	−	+
ABPC103	A-MuLV; prist.	−	−	−	−	+
ABPC105	A-MuLV; prist.	−	−	−	−	+
FLOPC78	Prist.	−	−	−	−	
SP2/0	Prist.	−	−	−	−	
TEPC1166	Prist.	−	−	−	−	
TEPC1174	Prist.	−	−	−	−	
TEPC2027	Prist.	−	−	−	−	
TEPC2241gl	Prist.	−	−	−	−	
TEPC2242gl	Prist.	−	−	−	−	
TEPC2243gl	Prist.	−	−	−	−	
TEPC2244gl	Prist.	−	−	−	−	
TEPC2245gl	Prist.	−	−	−	−	
TEPC2246gl	Prist.	−	−	−	−	
TEPC2247gl	Prist.	−	−	−	−	
TEPC2248gl	Prist.	−	−	−	−	
TEPC2250gl	Prist.	−	−	−	−	
TEPC2251gl	Prist.	−	−	−	−	
TEPC2268	Prist.	−	−	−	−	
J3PC2094	J3; prist.	−	−	−	−	
J3PC2105	J3; prist.	−	−	−	−	
J3PC2139	J3; prist.	−	−	−	+	
Macrophage-monocytic tumors						
McML2-1-1	*Myc*; Prist	−	−	−	−	
McML2-1-8	*Myc*; Prist	−	−	−	−	
McML2-2-2	*Myc*; Prist	−	−	−	−	
Sarcoma						
Meth A	Methylcholanthrene	+	ND	+	ND	

[a] prist. pristane; ND, not determined; +, positive for mutation; −, negative for mutation.

nature of this mutation, we reamplified DNA from this tumor and sequenced the exon 8 region (Fig. 2). A single A-G transition was found which results in an Arg-to-Gly substitution at residue 277 of mouse *p53*. Interestingly, substitutions at residue 277 of Arg to Ile have also been reported in mouse fibrosarcomas (18). Identical substitutions of Arg to Gly (residue 280) have also been identified in human breast and esophageal carcinomas (10).

A vast array of tumors have lost the ability to express wild-type *p53*, consistent with the fact that normal *p53* is a potent tumor suppressor (9, 10). Mutation of *p53* is common in Burkitt's lymphoma (33–60%) and B-cell acute lymphoblastic leukemia, type L3 (56%), both of which contain deregulated c-*myc* as a major genetic lesion (5–7). In a survey of Burkitt's lymphomas from a geographically distinct population, the frequency of *p53* mutation also appears to be about 60%.[6] In contrast, *p53* mutation is infrequent in other lymphoid neoplasms (5). Despite the apparent correlation between deregulated c-*myc* and *p53* mutation in human tumors, we have been unable to extend this observation to mouse plasmacytomas or monocytic tumors with deregulated c-*myc*.

The high incidence of *p53* mutation in Chinese and South African hepatocellular carcinomas is postulated to result from DNA damage caused by aflatoxin (19, 20). Interestingly, i.p. pristane provokes a chronic inflammatory response in susceptible BALB/c mice, which progresses from an oil granulomatous stage to a plasmacytoma. It is believed that this chronic inflammation may elicit the formation of DNA-damaging agents, such as oxygen radicals, from cells in the vicinity, *i.e.*, neutrophils and macrophages (21). The inefficient repair of this damage, for example, in the region 5′ of exon 1 of c-*myc*, appears to be a potential contributor to deregulated c-*myc* expression and possibly to plasmacytomagenesis (22). In spite of this highly mutagenic environment, *p53* does not appear to be affected, suggesting a specific lack of DNA damage, or efficient DNA repair.

The high frequency of *p53* mutation in Burkitt's lymphoma contrasts dramatically with that of the mouse plasmacytoma which is considered to be a more terminally differentiated B-cell neoplasm (1). Recent reports suggest that wild-type, unmutated *p53* may serve to facilitate normal differentiation in pre-B-cells (23, 24). This raises the interesting possibility that *p53* mutation may participate in enhancing the transformation process through a block to cellular differentiation.

[6] M. I. Gutierrez and K. Bhatia, manuscript in preparation.

References

1. Potter, M. Neoplastic development in B-lymphocytes. Carcinogenesis (Lond.), *11:* 1–13, 1990.
2. Mushinski, J. F. c-myc oncogene activation and chromosomal translocation in BALB/c plasmacytomas. *In:* G. Klein (ed.), Cellular Oncogene Activation, pp. 181–222. New York: Marcel Dekker, Inc., 1988.
3. Land, H., Parada, L. F., and Weinberg, R. A. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature (Lond.), *304:* 596–601, 1983.
4. Ohno, S., Migita, S., Wiener, F., Babonits, M., Klein, G., Mushinski, J. F., and Potter, M. Chromosomal translocations activating *myc* sequences and transduction of v-*abl* are critical events in the rapid induction of plasmacytomas by pristane and Abelson virus. J. Exp. Med., *159:* 1762–1777, 1984.
5. Gaidano, G., Ballerini, P., Gong, J. Z., Inghirami, G., Neri, A., Newcomb, E. W., Magrath, I. T., Knowles, D. M., and Dalla-Favera, R. *p53* mutations in human lymphoid malignancies: association with Burkitt lymphoma and chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. USA, *88:* 5413–5417, 1991.

6. Farrell, P. J., Allan, G. J., Shanahan, F., Vousden, K. H., and Crook, T. *p53* is frequently mutated in Burkitt's lymphoma. EMBO J., *10:* 2879–2887, 1991.
7. Wiman, K. G., Magnusson, K. P., Ramqvist, T., and Klein, G. Mutant *p53* detected in a majority of Burkitt lymphoma cell lines by monoclonal antibody Pab240. Oncogene, *6:* 1633–1639, 1991.
8. Ronen, D., Rotter, V., and Reisman, D. Expression from the murine *p53* promoter is mediated by factor binding to a downstream helix-loop-helix recognition motif. Proc. Natl. Acad. Sci. USA, *88:* 4128–4132, 1991.
9. Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Jr., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A., and Friend, S. H. Germ line *p53* mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science (Washington DC), *250:* 1233–1238, 1990.
10. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. *p53* mutations in human cancers. Science (Washington DC), *253:* 49–53, 1991.
11. Wolff, L., Mushinski, J. F., Shen-Ong, G. L. C., and Morse, H. B., III. A chronic inflammatory response: its role in supporting the development of c-*myb* and c-*myc* related promonocytic and monocytic tumors in BALB/c mice. J. Immunol., *141:* 681–689, 1988.
12. Mushinski, J. F., Bauer, S. R., Potter, M., and Reddy, E. P. Increased expression of *myc*-related oncogene mRNA characterizes most BALB/c plasmacytomas induced by pristane or Abelson murine leukemia virus. Proc. Natl. Acad. Sci. USA, *89:* 1073–1077, 1983.
13. Kurie, J. M., Morse, H. C., III, Principato, M. A., Wax, J. S., Troppmair, J., Rapp, U. R., Potter, M., and Mushinski, J. F. v-*myc* and v-*raf* act synergistically to induce B-cell tumors in pristane-primed adult BALB/c mice. Oncogene, *5:* 577–582, 1990.
14. Bienz, B., Zakut-Houri, R., Givol, D., and Oren, M. Analysis of the gene coding for the murine cellular tumor antigen p53. EMBO J., *3:* 2179–2183, 1984.
15. Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K., and Sekiya, T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc. Natl. Acad. Sci. USA, *86:* 2766–2770, 1989.
16. Eliyahu, D., Goldfinger, N., Pinhasi-Kimhi, O., Shaulsky, G., Skurnik, Y., Arai, N., Rotter, V., and Oren, M. MethA fibrosarcoma cells express two transforming mutant p53 species. Oncogene, *3:* 313–321, 1988.
17. Zakut-Houri, R., Oren, M., Bienz, B., Lavie, V., Hazum, S., and Givol, D. A single gene and a pseudogene for the cellular tumour antigen p53. Nature (Lond.), *306:* 594–597, 1983.
18. Halevy, O., Rodel, J., Peled, A. and Oren, M. Frequent p53 mutations in chemically induced murine fibrosarcomas. Oncogene, *6:* 1593–1600, 1991.
19. Hsu, I. C., Metcalf, R. A., Sun, T., Welsh, J. A., Wang, N. J., and Harris, C. C. Mutational hotspot in the *p53* gene in human hepatocellular carcinomas. Nature (Lond.), *350:* 427–428, 1991.
20. Bressac, B., Kew, M., Wands, J., and Ozturk, M. Selective G to T mutation of *p53* gene in hepatocellular carcinoma from southern Africa. Nature (Lond.), *350:* 429–431, 1991.
21. Potter, M., Wax, J. S., Anderson, A. O., and Nordan, R. P. Inhibition of plasmacytoma development in BALB/c mice by indomethacin. J. Exp. Med., *161:* 996–1001, 1985.
22. Beecham, E. J., Mushinski, J. F., Shacter, E., Potter, M., and Bohr, V. A. DNA repair in the c-*myc* proto-oncogene locus: possible involvement in susceptibility or resistance to plasmacytoma induction in BALB/c mice. Mol. Cell. Biol., *11:* 3095–3104, 1991.
23. Shaulsky, G., Goldfinger, N., and Rotter, V. Alterations in tumor development *in vivo* mediated by expression of wild type or mutant p53 protein. Cancer Res., *51:* 5232–5237, 1991.
24. Shaulsky, G., Goldfinger, N., Peled, P., and Rotter, V. Involvement of wild-type p53 in pre-B-cell differentiation *in vitro*. Proc. Natl. Acad. Sci. USA, *88:* 8982–8986, 1991.