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Calorie restriction delays spontaneous tumorigenesis in p53-knockout transgenic mice

STEPHEN D. HURSTING, SUSAN N. PERKINS, AND JAMES M. PHANG*

Laboratory of Nutritional and Molecular Regulation, Division of Cancer Prevention and Control, National Cancer Institute–Frederick Cancer Research and Development Center, Frederick, MD 21702

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ABSTRACT **Transgenic mice with both alleles of the p53 tumor suppressor gene (frequently mutated in human tumors) knocked out by gene targeting provide a potentially useful tumorigenesis model because these mice rapidly develop spontaneous tumors. To determine whether tumorigenesis in p53-knockout mice is sensitive to experimental manipulation, tumor development in response to calorie restriction (CR; a potent inhibitor of rodent tumors) was evaluated. Tumor development was monitored for 48 weeks in male nullizygous p53-knockout and wild-type littermate mice (28–30 per treatment group) fed ad libitum (AL) or restricted to 60% of AL carbohydrate calorie intake. CR:p53-knockout mice (median survival = 25 weeks) experienced a delay in tumor onset and subsequent mortality ($P = 0.0002$) relative to AL:p53-knockout mice (median survival = 16 weeks). Tumor development and mortality in wild-type littermates on either diet treatment were <4% through 48 weeks. Cell cycle analyses were performed on splenocytes from p53-knockout mice and wild-type littermates after 4 weeks of AL feeding or CR (5 per group). The percentage of splenocytes in S phase of the cell cycle was 3-fold higher for p53-knockout mice than wild-type mice ($P < 0.001$), and CR reduced the percentage of S-phase splenocytes in both p53-knockout and wild-type mice ($P = 0.012$). These data demonstrate that tumor development in p53-knockout mice genetically predisposed to tumors can be delayed by CR (possibly via cell cycle modulation) and suggest that these mice provide a very useful model of spontaneous tumorigenesis.**

Mutation of the p53 tumor suppressor gene is the most frequently observed genetic lesion in human cancer (1, 2). Over 50% of all human tumors examined to date have identifiable p53 gene point mutations or deletions (3). In addition, p53 mutations are associated with the Li–Fraumeni syndrome, a familial autosomal dominant disease characterized by increased susceptibility to a variety of tumors (4).

The accumulation of p53 protein in response to DNA damage induces a cell cycle arrest in the G_1 phase that appears to allow time for repair of damaged DNA prior to entry into S phase (5, 6). Acting as a transcription factor, p53 turns on the expression of WAF1/Cip1, the 21-kilodalton protein product of which can mediate the p53-induced arrest of the cell cycle (7). If the repair process in damaged cells is unsuccessful, p53 can trigger apoptotic cell death, at least in some cell types (8). Cells in which p53 is inactivated lack this cell cycle checkpoint control for DNA damage and are genetically less stable (9). The viability of mature nullizygous p53 "knockout" ($p53^{-/-}$) mice, with both alleles of the p53 gene inactivated by gene targeting techniques (10), implies that the p53 protein is unnecessary for normal growth and development. However, $p53^{-/-}$ mice are highly susceptible at an early age to a variety of spontaneous tumors (10), a further confirmation of the tumor-suppressing role of p53.

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Given the frequency of p53 mutations in human tumors, $p53^{-/-}$ mice provide an attractive tumorigenesis model since tumor development in these mice is rapid and spontaneous. The purpose of the present study was to determine if tumorigenesis in $p53^{-/-}$ mice is responsive to dietary manipulation, specifically to calorie restriction (CR), a well-documented inhibitor of tumors in several species and a multiplicity of tumor types (11–13). In addition, because CR has been shown to decrease mitotic rates in a variety of normal and neoplastic tissues (14, 15) and because $p53^{-/-}$ mice primarily develop hematopoietic tumors with splenic involvement, the effect of CR on splenocyte cell cycle kinetics in $p53^{-/-}$ and wild-type littermate ($p53^{+/+}$) mice was investigated.

MATERIALS AND METHODS

Animals and Diets. Weanling (4–5 weeks old) male $p53^{-/-}$ and $p53^{+/+}$ mice (GenPharm, Mountain View, CA) were individually housed in polycarbonate cages on hardwood bedding and maintained on a 12-hr light/dark cycle at 24°C. The $p53^{-/-}$ mice and their $p53^{+/+}$ littermates had the same genetic background (≈94% C57BL/6 and ≈6% 129/Sv), differing only at the p53 locus. Following a 2-week acclimation period after receipt, during which time all mice were fed AIN-76A semipurified diet (Bio-Serve, Frenchtown, NJ) ad libitum (AL), the mice were randomized to one of two dietary treatment groups: (*i*) AL, which received AIN-76A diet AL or (*ii*) CR, which received daily aliquots of a specially modified AIN-76A diet restricted to 60% of AL consumption. The compositions of both diets are summarized in Table 1. The CR diet was formulated such that the reduction in calories was entirely from the carbohydrate components in the diet, with all other components isonutrient relative to the AL group when consumed at 60% of the average intake of the AL mice. Food intakes and body weights were recorded weekly and all mice were observed daily for clinical signs of ill health. Feed was administered to both groups as 1-g dustless precision pellets in standard feeders and was provided to the AL mice in weekly aliquots and to CR mice in daily aliquots. The daily amount of feed provided to CR mice for a given week was based on 60% of the average daily feed intake of the AL mice for the previous week. All mice also received distilled water AL.

Spontaneous Tumorigenesis Study. Fifty-eight $p53^{-/-}$ mice and 60 $p53^{+/+}$ mice were randomly allocated to AL or CR diet regimens (30 per group, except the CR: $p53^{-/-}$ group, which had 28 mice) and observed and palpated daily to assess spontaneous tumor development. Moribund mice were killed with CO_2. All animals that were killed or found dead were

Abbreviations: CR, calorie restriction; AL, ad libitum; $p53^{-/-}$, nullizygous p53-knockout; $p53^{+/+}$, wild-type p53.

*To whom reprint requests should be addressed at: Laboratory of Nutritional and Molecular Regulation, Building 560, Room 12–48, National Cancer Institute–Frederick Cancer Research and Development Center, Frederick, MD 21702.

Table 1. Diet composition

Component	AL-fed diet, g/kg of diet	CR diet	
		g/kg of diet	g/0.6 kg of diet*
Casein	200	333.3	200
DL-Methionine	3	5	3
AIN 76A vitamin mix	10	16.7	10
Choline bitartrate	2	3.3	2
AIN 76A mineral mix	35	58.3	35
Sucrose	250	167	100
Corn starch	150	83	50
Dextrose	250	167	100
Fiber	50	83.3	50
Corn oil	50	83.3	50

*CR mice received the CR diet in daily aliquots equal to 60% of the average daily food intake of AL-fed mice.

subjected to necropsy and representative tissues were fixed in 10% neutral buffered formalin. Tissues were dehydrated through ascending grades of alcohol, cleared in xylene, and infiltrated with paraffin with an IMS LX300 tissue processor (Hacker Instruments, Fairfield, NJ). The paraffin blocks were sectioned (4–6 μm) on a Sergipath A5325 rotary microtome (Medical Industries, Richmond, IL). Sections were stained with hematoxylin/eosin and histopathologically analyzed to determine cause of death and tumor types. Mean body weights were compared between treatment groups using one-way analysis of variance, and tumor types were compared using a Yates-corrected χ^2 test (16). Differences in survival were compared using Cox proportional hazards analysis (17).

Splenocyte Cell Cycle Analysis. An additional group of 10 male $p53^{-/-}$ mice and 10 male $p53^{+/+}$ littermates was randomized to AL or CR regimens (5 per group). These mice were killed with CO_2 following 4 weeks of diet treatment and splenocytes were isolated as described (9). In brief, spleens were aseptically removed and placed in cold RPMI 1640 medium (GIBCO). Individual spleens were macerated and filtered through sterile gauze. Suspensions of single cells were made by passing the filtrate through a 25-gauge needle with a 20-ml syringe. Erythrocytes in the splenocyte cell suspension were lysed by incubation with red blood cell lysing buffer (Sigma) for 1 min. Following three washes in complete RPMI 1640 medium (supplemented with 10% fetal bovine serum, 100 units of penicillin per ml, 100 μg of streptomycin per ml, 10 mM Hepes buffer, 2 mM L-glutamine, and 1 mM sodium pyruvate; all from GIBCO), each splenocyte sample was counted with a Coulter ZM cell counter and cell concentrations were adjusted to 1×10^7 cells per ml in the complete RPMI medium. For the Thornthwaite procedure (18), aliquots (100 μl) of each splenocyte sample were mixed with 400 μl of complete medium and 500 μl of Thornthwaite buffer containing 50 mg of propidium iodide per liter (Sigma) and 7.5 units of RNase One (Promega). These stained samples were stored at 4°C for 24–48 hr prior to cell cycle analysis on a Coulter Epics flow cytometer. The percentage of cells in S phase of the cell cycle in each sample was determined by flow cytometric analysis, and comparisons were made between treatment groups by one- and two-way analysis of variance procedures and Duncan's multiple comparison procedure (16).

RESULTS

The growth curves of the $p53^{-/-}$ mice on either dietary regimen were virtually identical to the growth curves of the $p53^{+/+}$ mice through the first 15 weeks of study (Fig. 1). The

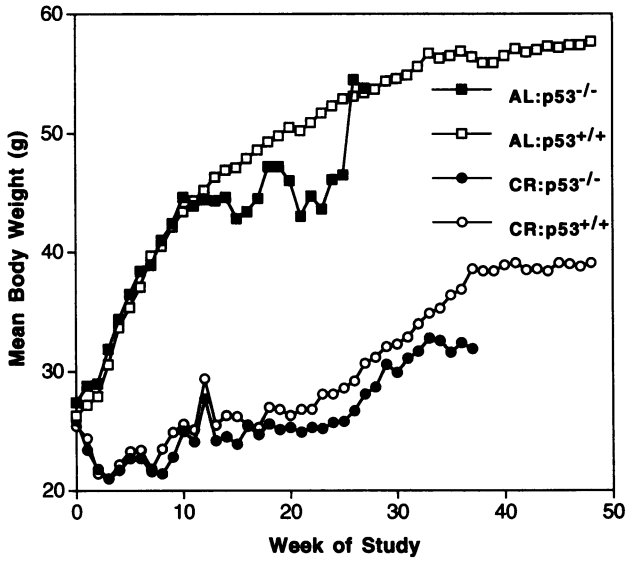

FIG. 1. Mean body weights by week of study of p53-knockout ($p53^{-/-}$) and wild-type ($p53^{+/+}$) mice fed AL or calorie-restricted (CR; 28–30 mice per treatment group).

average body weights of the $p53^{-/-}$ mice showed some fluctuations after this time due to the onset of tumor development in a large proportion of the group. CR mice, regardless of p53 status, were significantly smaller ($P < 0.01$) than AL mice throughout the study period.

Marked differences were seen in the survival curves for the respective treatment groups (Fig. 2). As expected, very little tumor development or mortality was observed in $p53^{+/+}$ mice on either diet treatment through the 48-week study period. In contrast, all $p53^{-/-}$ mice were dead by 37 weeks of study. However, CR:$p53^{-/-}$ mice showed a statistically significant delay in tumor-related mortality relative to AL:$p53^{-/-}$ mice ($P = 0.0002$), based on Cox proportional hazards analysis (17). Median time to death was 16 weeks for the AL:$p53^{-/-}$ mice, and all mice in this group were dead by 28 weeks of study. For the CR:$p53^{-/-}$ mice, the median time to death increased to 25 weeks, and the final mice in this group

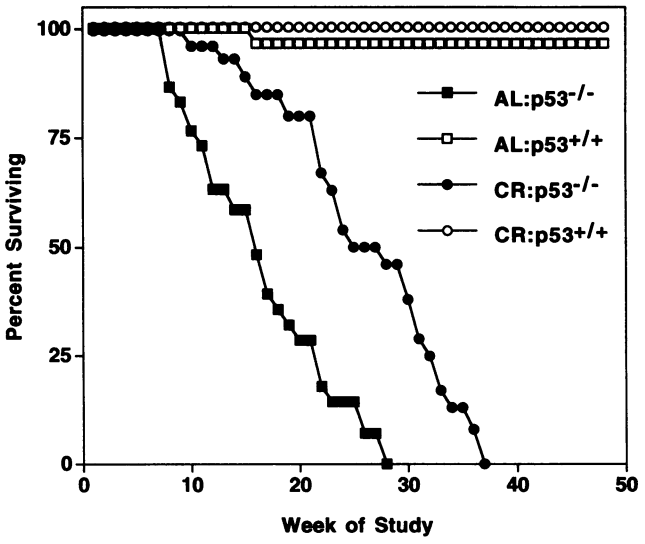

FIG. 2. Percent survival of $p53^{-/-}$ and $p53^{+/+}$ mice fed AL or calorie-restricted (CR; 28–30 mice per treatment group) through 48 weeks of study. Based on Cox proportional hazards analysis (17), CR:$p53^{-/-}$ mice showed a statistically significant delay in mortality due to spontaneous tumor development ($P = 0.0002$).

Table 2. Tumor types in p53-knockout mice

Case	TTD*	Histological type†	Anatomic site‡	Other tumors (site)
AL1	46	Lymphoma	Generalized	
AL2	46	Lymphoma	Generalized	
AL3	46	Astrocytoma	Brain	
AL4	51	Hemangiosarcoma	Muscle; lung	
AL5	52	Lymphoma	Generalized	
AL6	59	Hemangiosarcoma	Subcutis	
AL7	59	Lymphoma	Generalized	Thymoma
AL8	72	Alveolar carcinoma	Lung	
AL9	73	Lymphoma	Generalized	Squamous cell papilloma (stomach)
AL10	73	Hemangiosarcoma	Muscle; lung	
AL11	77	Lymphoma	Generalized	Thymoma
AL12	80	‡		
AL13	86	Lymphoma	Generalized	
AL14	100	Lymphoma	Generalized	Carcinoma (alveolar)
AL15	100	Sarcoma	Leg	
AL16	100	Neuroblastoma	Olfactory	Lymphoma (spleen)
AL17	107	Lymphoma	Generalized	Sarcoma (prostate)
AL18	107	‡		
AL19	108	‡		Thymoma
AL20	115	‡		Adenoma (cecum)
AL21	122	Lymphoma	Generalized	Sarcoma (leg); thymoma
AL22	128	Neuroblastoma	Cauda equina	
AL23	142	Lymphoma	Generalized	
AL24	143	Carcinoma	Lymph node	
AL25	143	Lymphoma	Generalized	
AL26	156	Hemangiosarcoma	Lymph nodes	Leiomyosarcoma (stomach)
AL27	170	Lymphoma	Thymus	Granulocytic leukemia (generalized)
AL28	171	Lymphoma	Generalized	
AL29	185	Lymphoma	Generalized	Carcinoma (liver); sarcoma (histiocytic)
AL30	185	Lymphoma	Generalized	
CR1	43	‡		
CR2	60	Lymphoma	Generalized	
CR3	81	Lymphoma	Generalized	
CR4	93	Lymphoma	Generalized	
CR5	100	Lymphoma	Generalized	
CR6	107	‡		
CR7	115	‡		
CR8	121	Lymphoma	Generalized	
CR9	141	‡		
CR10	142	Sarcoma	Leg	Lymphoma (generalized); thymoma
CR11	143	‡		Sarcoma (lymph node)
CR12	144	Lymphoma	Generalized	
CR13	148	Hemangiosarcoma	Lung	Thymoma
CR14	150	Lymphoma	Generalized	
CR15	156	Lymphoma	Generalized	
CR16	156	Sarcoma	Body wall	
CR17	165	Lymphoma	Generalized	
CR18	189	Lymphoma	Generalized	Hemangiosarcoma (spleen)
CR19	199	Pheochromocytoma	Adrenal	
CR20	200	Lymphoma	Generalized	
CR21	207	Lymphoma	Generalized	
CR22	211	Lymphoma	Generalized	
CR23	217	Lymphoma	Generalized	Carcinoma (nose)
CR24	221	Hemangiosarcoma	Muscle	Thymoma; sarcoma (body wall)
CR25	225	Myeloid leukemia	Generalized	Lymphoma (thymus)
				Lymphoma (thymus); hemangiosarcoma (muscle); neuroblastoma (olfactory); carcinoma (kidney)
CR26	232	Granulocytic leukemia	Generalized	
CR27	249	Lymphoma	Generalized	
CR28	252	Lymphoma	Generalized	Hemangiosarcoma (liver)

*TTD, time to death (days).
†Histological type and anatomic site of tumor that caused death.
‡Non-tumor cause of death.

survived until 37 weeks of study. Thus, a dietary manipulation (i.e., CR) delayed tumor onset in $p53^{-/-}$ mice genetically predestined to an early death due to spontaneous tumor development.

Although the CR regimen markedly altered the time course of spontaneous tumorigenesis in $p53^{-/-}$ mice, it had no statistically significant effect on the types of malignant tumors developed by these animals (Tables 2 and 3). Lymphomas were the most commonly observed tumors; 61% of tumor-bearing AL:$p53^{-/-}$ mice and 79% of tumor-bearing CR:$p53^{-/-}$ mice developed malignant lymphoma. The majority of lymphomas occurring in $p53^{-/-}$ mice on either diet treatment were of lymphoblastic origin, although 25–30% were of immunoblastic or follicular center cell origin and an additional 15–30% were pleomorphic (morphologic evidence of both T- and B-cell elements in the same animal). Sarcomas were observed in 32% of tumor-bearing AL:$p53^{-/-}$ mice and 38% of CR:$p53^{-/-}$ mice. Most of the sarcomas occurring in $p53^{-/-}$ mice on either diet treatment group were hemangiosarcomas or anaplastic sarcomas in the subcutis or body wall. A number of other tumor types were less frequently observed, including neuroblastomas, leukemias, carcinomas (hepatic, alveolar, lymph node, renal, and nasal), thymomas, an adrenal pheochromocytoma, a squamous cell papilloma in the nonglandular stomach, a cecal adenoma, and an astrocytoma. CR also had no effect on tumor burden; ≈35% of tumor-bearing $p53^{-/-}$ mice on either dietary treatment developed multiple tumors. In addition, 88% of malignant tumor-bearing AL:$p53^{-/-}$ mice and 92% of CR:$p53^{-/-}$ mice had multiple metastases.

The major cause of death in the $p53^{-/-}$ mice on either dietary treatment was malignant tumor (Tables 2 and 3). Twenty-six AL:$p53^{-/-}$ mice died from malignant tumor development and one died from intussusception due to a cecal adenoma. The cause of death for the three remaining mice in this group appeared to be infection, based on histopathologic evidence. Twenty-three CR:$p53^{-/-}$ mice died from malignant tumor development. Of the remaining five CR:$p53^{-/-}$ mice, one died from intestinal blockage due to intussusception; the cause of death could not be determined for the other four mice. Among the $p53^{+/+}$ mice, one AL-fed animal died from spontaneous lymphoma development during the 48-week study period. Three other AL:$p53^{+/+}$ mice died tumor-free; one displayed histopathologic evidence of infection, while the other two died of undetermined causes. None of the CR:$p53^{+/+}$ mice developed tumors through the 48 weeks of study, although one CR:$p53^{+/+}$ mouse died of undetermined causes.

Table 3. Summary of tumor development in AL-fed or CR p53-knockout mice

	AL		CR	
	%	No.	%	No.
Tumor type*				
Lymphoma	61	17/28	79	19/24
Lymphoblastic	29	8/28	41	10/24
Immunoblastic or follicular center cell	14	4/28	25	6/24
Pleomorphic	18	5/28	13	3/24
Sarcoma	32	9/28	38	9/24
Hemangiosarcoma	14	4/28	21	5/24
Other sarcomas	18	5/28	17	4/24
Other malignant tumors	29	8/28	21	5/24
Benign tumors	21	6/28	13	3/24
Multiple tumors†	36	10/28	38	9/24
Cause of death‡				
Malignant tumor	87	26/30	82	23/28
Nonmalignant death	13	4/30	18	5/28

Tumor types or causes of death were not statistically different between AL-fed or CR p53-knockout mice at $P < 0.05$.
*Percent of tumor-bearing animals.
†Percent of tumor-bearing animals with two or more tumors.
‡Percent of all mice in each group.

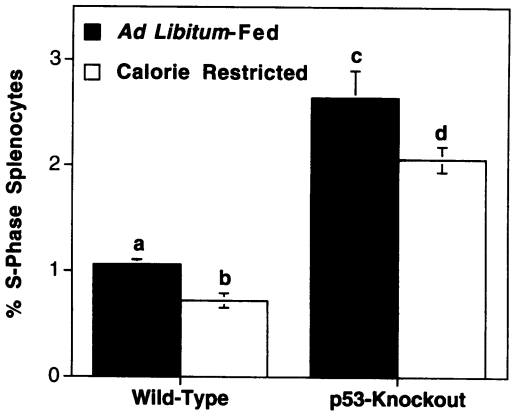

FIG. 3. Percent S-phase splenocytes from $p53^{-/-}$ and $p53^{+/+}$ mice fed AL or calorie-restricted (CR; 5 per treatment group). Cell cycle kinetics were evaluated by flow cytometry. Data are expressed as the mean percent (± SD) of splenocytes in S phase of the cell cycle. Values with different superscripts are statistically different at $P < 0.05$.

Splenocytes were analyzed by flow cytometry to evaluate the effect of p53 gene knockout in the $p53^{-/-}$ mice on splenocyte proliferation and to determine if CR can modulate the cell cycle in these mice. *Ex vivo* splenocytes were used because they are easily isolated and because the majority of tumors observed in the $p53^{-/-}$ mice were malignant lymphomas with splenic involvement. Splenocytes were isolated from five mice in each treatment group after 4 weeks of diet treatment; Fig. 3 shows the percentage of cells in S phase of the cell cycle. None of the mice used in these analyses had tumors, as determined by histopathologic analysis. The mean percentage of splenocytes in S phase was 22% lower in CR:$p53^{-/-}$ mice than AL:$p53^{-/-}$ mice ($P = 0.029$). Likewise, a 32% lower percentage of splenocytes was in S phase in the CR:$p53^{+/+}$ mice than in the AL:$p53^{+/+}$ mice ($P < 0.001$). Two-way analysis of variance revealed that the percentage of S-phase cells was higher for $p53^{-/-}$ mice than $p53^{+/+}$ mice ($P < 0.001$) and that CR significantly reduced the percentage of S-phase splenocytes ($P = 0.012$). A statistically significant interaction between p53 status and diet treatment was not observed ($P = 0.32$), indicating that CR modulated the cell cycle equivalently in $p53^{-/-}$ and $p53^{+/+}$ mice.

DISCUSSION

The rapid development of tumors and the sharp increase in mortality in our AL:$p53^{-/-}$ mice, beginning around the 10th week of study, demonstrated the extraordinary susceptibility of $p53^{-/-}$ mice to spontaneous tumorigenesis. In contrast, AL:$p53^{+/+}$ mice showed virtually no tumor development through 48 weeks of study. This rapid onset of tumor development in AL:$p53^{-/-}$ mice is consistent with a report showing that untreated $p53^{-/-}$ mice with a genetic background (≈75% C57BL/6 and ≈25% 129/Sv) similar to the mice used in this study develop normally but die of spontaneous tumors, particularly lymphomas or sarcomas, at an early age (10).

Numerous studies have shown that CR increases longevity and inhibits a variety of spontaneous or experimentally-induced tumors in rodents (11–13), including spontaneous lymphomas in C57BL/6 mice, the main background strain of the mice used in the present study (19). The results reported here indicate that CR can modulate spontaneous tumorigenesis even in $p53^{-/-}$ mice. The delayed onset of tumor

development observed in the CR:p53$^{-/-}$ mice, relative to AL:p53$^{-/-}$ mice, suggests that the wild-type p53 tumor suppressor gene is not an absolute requirement for the tumor-suppressive effects of CR. These data also demonstrate that a dietary perturbation (i.e., CR) can influence the outcome of a genomic liability such as the accelerated tumorigenesis displayed by p53-knockout mice.

The findings from our cell cycle studies may provide insights into the predisposition of p53$^{-/-}$ mice to spontaneous lymphoma development as well as the tumor-suppressing effects of CR. Lymphoid cells, which undergo programed DNA rearrangements of the immunoglobulin genes, appear to be particularly susceptible to the loss of p53 (20, 21). Although our cell cycle analyses are limited by small sample sizes (n = 5 mice per group) and incomplete analyses of cell cycle parameters (Thornthwaite procedure; ref. 18), the data suggest that splenocytes from AL:p53$^{-/-}$ mice have a higher percentage of S-phase cells relative to splenocytes from AL:p53$^{+/+}$ mice. This finding is consistent with a report that embryonic fibroblasts derived from p53$^{-/-}$ mice also have a higher percentage of cells in S phase than embryonic fibroblasts from p53$^{+/+}$ mice (20). Premature entry of lymphocytes into S phase before the correct gene rearrangements are completed could result in aberrant recombination events that are potentially mutagenic (20, 21). In addition, lymphocytes from p53$^{-/-}$ mice would lack the p53-mediated apoptotic pathway and might thus exhibit increased survival of lymphoid precursors with abnormal rearrangements (21). This could explain why inactivation of the p53 gene results in a high incidence of lymphoma in p53$^{-/-}$ mice derived from strains with a high background incidence of lymphoma, such as the predominantly C57BL/6 strains (this study; refs. 10 and 20) and a pure 129/Ola strain (21), as well as in p53$^{-/-}$ mice derived from 129/Sv mice, a strain with a low background incidence of lymphoma (20).

Our data also indicate that CR decreases (by 20–30%) the percentage of S-phase splenocytes in both p53$^{-/-}$ and p53$^{+/+}$ mice, consistent with previous reports of reduced mitotic activity in a variety of tissues in response to CR (14, 15). Elucidation of the mechanisms underlying the observed effects of CR on spontaneous tumor development and splenocyte cell cycle in p53$^{-/-}$ mice may reveal some important intervention targets that can be exploited in the development of cancer prevention strategies to counteract the increased risk of cancer seen with loss of p53 tumor suppressor gene function.

To our knowledge, there has been no previous report of an experimental treatment that suppresses tumor development in p53$^{-/-}$ mice, which (because of germ-line inactivation of the p53 gene) are genetically predisposed to early spontaneous tumorigenesis. The mechanistic pathways in p53$^{-/-}$ mice responsible for modulated tumor development should have relevance to human cancer, since mutations in the p53 gene are the most common genetic lesions observed in human tumors (1–3). In addition, tumor development in these mice is spontaneous, thus eliminating the need for carcinogens or other agents to induce tumorigenesis, and is rapid, with untreated p53$^{-/-}$ mice showing 50% mortality due to malignant tumors by about 5 months of age. Finally, the demonstration of a delay in tumor development in p53$^{-/-}$ mice in response to experimental manipulation suggests that these mice provide a useful model of spontaneous tumorigenesis.

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1. Levine, A. J., Momand, J. & Finlay, C. A. (1991) *Nature (London)* **351**, 453–456.
2. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C. C. (1991) *Science* **253**, 49–53.
3. Chang, F., Syrjanen, S., Kurvinen, K. & Syrjanen, K. (1993) *Am. J. Gastroenterol.* **88**, 174–186.
4. Srivastava, S., Zou, Z., Pirollo, K., Blattner, W. & Chang, E. H. (1990) *Nature (London)* **348**, 747–749.
5. Pietenpol, J. A. & Vogelstein, B. (1993) *Nature (London)* **365**, 17–18.
6. Kuerbitz, S. J., Plunkett, B. S., Walsh, W. V. & Kastan, M. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7491–7495.
7. El-Deiry, W. S., Harper, J. W., O'Connor, P. M., Velculescu, V. E., Canman, C. E., Jackman, J., Pietenpol, J. A., Burrell, M., Hill, D. E., Wang, Y., Wiman, K. G., Mercer, W. E., Kastan, M. B., Kohn, K. W., Elledge, S. J., Kinzler, K. W. & Vogelstein, B. (1994) *Cancer Res.* **54**, 1169–1174.
8. Yonisch-Rouach, E., Resnitzky, D., Lotem, J., Sachs, L., Kimchi, A. & Orren, M. (1991) *Nature (London)* **352**, 345–347.
9. Livingstone, L. R., White, A., Sprouse, J., Livanos, E., Jacks, T. & Tlsty, T. D. (1992) *Cell* **71**, 587–597.
10. Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Butel, J. & Bradley, A. (1992) *Nature (London)* **356**, 215–221.
11. Weindruch, R. & Walford, R. L. (1988) *The Retardation of Aging and Disease By Dietary Restriction* (Thomas, Springfield), pp. 73–229.
12. Boissonneault, G. (1991) in *Nutrition, Toxicity and Carcinogenesis*, ed. Rowland, I. (CRC Press, Boca Raton), pp. 413–437.
13. Hursting, S. D., Switzer, B. R., French, J. E. & Kari, F. W. (1993) *Cancer Res.* **53**, 2750–2757.
14. Lok, E., Scott, F. W., Mongeau, R., Nera, E. A., Malcolm, S. & Clayson D. B. (1990) *Cancer Lett.* **51**, 67–73.
15. Heller, T. D., Holt, P. R. & Richardson, A. (1990) *Gastroenterology* **98**, 387–391.
16. Kleinbaum, D. G., Kupper, L. L. & Muller K. E. (1988) *Applied Regression Analysis and Other Multivariable Methods* (Kent, Boston), pp. 341–385.
17. Thomas, D. G., Breslow, N. & Gart, J. J. (1977) *Comp. Biomed. Res.* **10**, 373–381.
18. Thornthwaite, J. T., Sugarbaker, E. V. & Temple, W. T. (1980) *Cytometry* **1**, 229–237.
19. Koizumi, A., Tsukada, M., Wada, Y., Masuda, H. & Weindruch, R. (1992) *J. Nutr.* **122**, 1446–1453.
20. Harvey, M., McArthur, M. J., Montgomery, C. A., Bradley, A. & Donehower, L. D. (1993) *FASEB J.* **7**, 938–943.
21. Purdie, C. A., Harrison, D. J., Peter, A., Dobbie, L., White, S., Howie, S. E. M., Salter, D. M., Bird, C. C., Wyllie, A. H., Hooper, M. L. & Clarke, A. R. (1994) *Oncogene* **9**, 603–609.