Diet and Cancer Prevention Studies in p53-Deficient Mice

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ABSTRACT Progress in mechanism-based cancer prevention research may be facilitated by the use of animal models displaying specific genetic susceptibilities for cancer such as mice deficient in the p53 tumor suppressor gene, the most frequently altered gene in human cancer. We observed in p53-knockout (p53-/-) mice that calorie restriction (CR; 60% of the control group’s intake of carbohydrate energy) increased the latency of spontaneous tumor development (mostly lymphomas) ~75%, decreased serum insulin-like growth factor (IGF)-1 and leptin levels, significantly slowed thymocyte cell cycle traverse and induced apoptosis in immature thymocytes. In heterozygous p53-deficient (p53 +/-) mice, CR and 1 d/wk of food deprivation each significantly delayed spontaneous tumor development (a mix of lymphomas, sarcomas and epithelial tumors) and decreased serum IGF-1 and leptin levels even when begun late in life. We have also developed a rapid and relevant p53 +/- mouse mammary tumor model by crossing p53-deficient mice with MMTV-Wnt-1 transgenic mice, and found that CR and 1 d/wk food deprivation significantly increased mammary tumor latency (greater than twofold) and reduced the mean serum IGF-1 and leptin levels to ~50% of that of control mice (P < 0.0001). In addition, fluasterone, fenretinide and soy each delayed tumor development but had little effect on IGF-1 or leptin levels. We have capitalized on the susceptibility of p53 +/- mice to chronic, low dose, aromatic amine-induced bladder carcinogenesis to develop a useful model for evaluating bladder cancer prevention approaches such as cyclooxygenase-2 inhibition. As demonstrated by these examples, mice with specific (and human-like) genetic susceptibilities for cancer provide powerful new tools for testing and characterizing interventions that may inhibit the process of carcinogenesis in humans.


KEY WORDS: • nutrition • chemoprevention • transgenic animals • calorie restriction • insulin-like growth factor-1 • leptin

The recent development of mouse strains with carcinogenesis-related genes overexpressed or inactivated provides investigators with new models for studying the carcinogenesis process and for testing preventive strategies that can offset highly relevant genetic susceptibilities to cancer in humans (1). Our work has focused on using mice deficient in the p53 tumor suppressor gene to ask the following question: can we offset increased cancer risk due to a genetic lesion such as loss of p53 suppressor activity by preventive (particularly nutritional) approaches? Mutation of the p53 tumor suppressor gene is the most frequently observed genetic lesion in human cancer; >50% of all human tumors examined to date have identifiable p53 gene point mutations or deletions (2). Donehower et al. (3) first reported in 1992 that homozygous p53-knockout (p53-/-) mice were viable but highly susceptible to spontaneous tumorigenesis (particularly lymphomas) at an early age. These p53-deficient mice have been useful tools for studying the role of p53 in carcinogenesis. For example, in response to the two-stage skin carcinogenesis protocol, p53-/- mice, relative to wild-type (p53+/+) mice, showed no difference in benign papilloma formation but did display greatly accelerated progression to malignant carcinomas (4). Furthermore, the carcinomas formed in the p53-/- mice showed higher indices of malignancy as measured by histopathology, further confirming the importance of p53 loss in acceleration of tumor progression. These mice also provide an attractive and potentially relevant tumorigenesis model for studying cancer prevention strategies, given the frequency of p53 mutations in human tumors and the rapidity with which spontaneous tumors develop in these mice. The purpose of this article is to provide a summary of studies to date that have used p53-deficient models for cancer prevention studies.

Prevention studies in p53-/- mice

We evaluated the ability of several dietary and chemopreventive interventions to offset the increased susceptibility of p53-/- mice to spontaneous tumorigenesis (5–8). We chose...
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calorie restriction (CR),3 the most potent and broad acting dietary perturbation of carcinogenesis (9), as our initial proof of principle that a dietary intervention could influence tumorigenesis in mice predestined to develop tumors because of a lack of the p53 tumor suppressor. CR reduces cell proliferation in most tissues studied (10). This decreased rate of DNA replication in normal cells in response to CR may make those cells less susceptible to mutations induced by carcinogens as well as suppress the progression of preneoplastic cells. In p53-/− mice, CR (60% of the control group’s intake of carbohydrate energy) increased the latency of spontaneous tumor development (mostly lymphomas) by ~75% and significantly slowed thymocyte and splenocyte cell cycle traverse (5). The time to tumor onset in these mice is p53 dependent, with most p53-/− mice developing and dying from spontaneous tumors by ~6 mo of age compared with nearly 2 y of age for p53+/+ mice. However, the highly significant tumor-delaying effect of CR, relative to ad libitum food consumption, was similar in both p53-/− and p53+/+ mice, indicating that the mechanisms underlying CR may be p53 independent (6).

We also found that several nutritional and chemopreventive agents could influence tumorigenesis in this model. Perhaps the most striking effect was with the chemopreventive steroid dehydroepiandrosterone (DHEA; 0.3% in the diet), which significantly delayed spontaneous tumorigenesis in p53-/− mice and in particular suppressed lymphoma development (7). The DHEA analog 16α-fluoro-5-androsten-17-one (fluasterone; 0.15% in the diet) also suppressed spontaneous lymphoma development and lengthened survival in p53-/− mice (8). Taken together, these findings clearly demonstrate that the increased susceptibility to cancer as a result of a genetic lesion, such as loss of p53 tumor suppressor function, may be offset, at least in part, by preventive approaches.

The p53-/− mice have also been useful for elucidating the mechanism of action underlying the tumor-inhibitory effects of CR and the chemopreventive steroids. For example, the antitumor effect of DHEA (or its fluorinated analog fluasterone) in p53-/− mice is independent of it effects on food intake or on nucleotide pool levels (7), as was suggested previously in other models (11). Wang et al. (12) showed that both CR and DHEA decreased thymocyte proliferative rates, and Poetschke et al. (13) showed that CR, DHEA and fluasterone each slowed thymocyte cell cycle progression, induced apoptosis in the lymphoma-susceptible subpopulation of immature thymocytes and (particularly the steroids) blocked thymocyte maturation. The apoptosis-inducing effects of the chemopreventive steroids appeared to be mediated by decreased Bcl-2 gene expression. The effects of CR on apoptosis were independent of the Bcl-2/Bax apoptotic regulatory pathway. However, CR (but not the steroids) significantly reduces circulating insulin-like growth factor (IGF)-1 levels (Hursting, S. D. and Perkins, S. N., National Cancer Institute, unpublished observations, 2001), which as suggested by Dunn et al. (14), may be responsible for the apoptotic-inducing effects of CR. Both CR and the chemopreventive steroids also decrease serum leptin levels (Hursting, S. N. and Perkins, S. D., unpublished observations, 2001). Leptin, the so-called fat hormone, has been shown to act as a proinflammatory cytokine (15), a proangiogenic factor (16) and also as an apoptotic regulator in certain cell types (17); this reduction in leptin levels may therefore also contribute to the effects of CR. In addition, Mei et al. (18) showed that CR, DHEA and fluasterone each suppressed nitric oxide levels and down-regulated nitric oxide synthetase expression. The roles of IGF-1, leptin and nitric oxide and other inflammatory components in the anticancer effects of CR in p53-/− mice are currently being further characterized.

Prevention studies in p53+/− mice

Heterozygous p53-knockout (p53+/−) mice, with only one p53 allele inactivated, have some analogy to humans susceptible to heritable forms of cancer because of decreased p53 gene dosage, such as individuals with Li-Fraumeni syndrome (19). The spontaneous tumors that most frequently occur in p53+/− mice (hematopoietic neoplasias and osteosarcomas) are similarly observed in humans with Li-Fraumeni syndrome. However, the two most common epithelial tumors observed in Li-Fraumeni patients (lung tumors in men and breast tumors in women) are infrequent in the mice. Tumor latency in p53+/− mice (median survival ~18 mo) is reduced relative to p53+/+ mice (median survival ~26 mo) although it is much longer than in p53-/− mice (median survival ~6 mo). CR and food deprivation for 1 d/wk both significantly delayed spontaneous tumor development (mainly lymphomas and various sarcomas) in male p53+/− mice, and fluasterone suppressed the development of spontaneous tumors (mainly osteosarcomas) in female p53+/− mice, even when interventions were started during adulthood (at ages 9–12 mo; Hursting, S.D. and Perkins, S.N., unpublished observations, 2001).

Although p53+/− mice have low rates of spontaneous tumorigenesis up to age 12 mo (20), they do display increased susceptibility to chemically induced tumor development relative to wild-type mice. p-Cresidine–induced bladder tumors (19), dimethylsulfoxamide-induced liver tumors (20), nitrosomethyleurea-induced lymphomas (Hursting, S.D. and Perkins, S.N., unpublished observations, 2001), azoxymethane–induced aberrant crypt foci and colon tumors (21), and radiation-induced lymphomas and sarcomas (22) all appear significantly earlier in p53+/− mice than in similarly treated p53+/+ mice. Malignant progression of DMBA-initiated, 12-O-tetradecanoyl phorbol-13-acetate–promoted skin papillomas occurred much more quickly in p53+/− mice than in p53+/+ mice (4). These findings suggest that p53+/− mice are more sensitive to several classes of mutagenic carcinogens than are p53+/+ mice and appear to be susceptible to at least some low dose, chronic carcinogen regimens that more closely mimic human exposures. Thus, these mice have tremendous potential for developing models facilitating the study of gene–environment interactions relevant to human cancer prevention.

Using the p-cresidine–induced bladder tumor model in male p53+/− mice, Dunn et al. (14) showed that CR (started after tumors had formed) could suppress bladder tumor progression. Furthermore, IGF-1 appeared to mediate the CR effect because restoration of IGF-1 serum levels in CR mice via osmotic pump infusion reversed the CR effect. We (23) previously reported a similar finding of a mediating role for IGF-1 in the anticancer effect of CR using a Fischer rat leukemia model. We have also evaluated the possible preventive effects of moderate CR (80% of control energy intake) along with the synthetic retinoid fenretinide and the nonsteroidal anti-inflammatory drug indomethacin in the p-cresidine bladder model in male p53+/− mice. In our study, the p-cresidine exposure and preventive regimens were begun simultaneously (when mice were 6 wk of age) and continued for 24 wk. CR induced a modest, nonsignificant reduction in bladder tumor incidence but suppressed the growth of p-cresidine–induced tumors, whereas indomethacin decreased bladder tumor inci-

3 Abbreviations used: DHEA, dehydroepiandrosterone; CR, calorie restriction; fluasterone, 16α-fluoro-5-androsten-17-one; IGF, insulin-like growth factor.
P53-deficient mammary tumor model

We have been characterizing a rapid and spontaneous p53+/- mouse mammary tumor model developed by crossing p53+/- mice with MMTV-Wnt-1 transgenic mice. In these mice, CR, 1 d/wk food deprivation, the synthetic retinoid fenretinide, the chemopreventive steroid fluasterone and a high soy diet each delay spontaneous mammary tumor development (25,26). Mammary tumors from these mice are estrogen-receptor positive, overexpress cyclooxygenase-2 and show reduced BRCA-1 expression, suggesting that this model, which involves alterations in two critical carcinogenesis pathways, may be highly relevant for the development of breast cancer prevention strategies.

In summary, carcinogen-induced models of cancer in rodents have been crucial to advancing our understanding of the neoplastic process, and recent progress in the fields of toxicology, pathology and molecular carcinogenesis has revealed multiple targets for the nutritional modulation and chemoprevention of cancer. We must now capitalize on the availability of new tools such as transgenic mice to identify additional targets that can be modulated and make important progress toward one of the major goals in contemporary cancer research, i.e., the development of effective mechanism-based strategies for preventing human cancer. Successful attainment of this goal will require the integration of the very best science from multiple levels of investigation, including clinical and epidemiologic research, animal studies and basic molecular and cellular biologic research. In our view, the animal model studies play a critical central role in this endeavor. For example, animal studies are required to confirm (under controlled experimental conditions) potential leads from human studies that show associations between certain risk factors (both protective and harmful) and cancer risk. In addition, preclinical studies are critical in translating basic mechanistic findings from the bench to the clinic or population. Thus, the development and characterization of highly relevant animal models will greatly facilitate future progress in cancer prevention research. We have discussed examples of cancer prevention studies that have used p53-deficient mouse models. Taken together, these examples clearly indicate that mice with specific (and human-like) genetic susceptibilities for cancer provide powerful new tools for testing interventions that may inhibit the process of carcinogenesis in humans.

LITERATURE CITED