

# Nutrition and Gene Regulation

## Diet-Gene Interactions in p53-Deficient Mice: Insulin-like Growth Factor-1 as a Mechanistic Target<sup>1</sup>

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**ABSTRACT** Progress in cancer prevention research is being facilitated by the use of animal models displaying specific genetic susceptibilities for cancer, such as mice deficient in one (+/−) or both (−/−) alleles of the p53 tumor suppressor gene. Our lab, which focuses on nutrition (particularly energy balance/obesity) and molecular carcinogenesis, has shown in p53−/− mice that calorie restriction (CR) increases the latency of spontaneous tumor development (mostly lymphomas) ~75%, decreases serum insulin-like growth factor (IGF)-1 and leptin levels, and induces apoptosis in immature (lymphoma-susceptible) thymocytes. In heterozygous p53-deficient (p53+/−) mice, CR and a one day/wk fast each significantly delay spontaneous tumor development (a mix of lymphomas, sarcomas, and epithelial tumors) and decreases serum IGF-1 and leptin levels, even when begun late in life. We are presently comparing and combining CR and exercise (treadmill and running wheel) to further elucidate the relationships between energy balance, p53, and tumorigenesis in these models. Furthermore, we have capitalized on the susceptibility of p53+/− mice to chronic, low-dose aromatic amine-induced bladder carcinogenesis to develop a model for evaluating bladder cancer prevention approaches. Using this model, we have established that IGF-1 mediates many of the anti-cancer effects of CR. We are currently conducting oligonucleotide microarray studies to further characterize diet-gene interactions underlying the anti-cancer effects of CR and to determine which of the CR-responsive genes are IGF-1 dependent. *J. Nutr.* 134: 2482S–2486S, 2004.

**KEY WORDS:** • nutrition • chemoprevention • transgenics • calorie restriction  
• insulin-like growth factor-1 • leptin

Cancer is thought to arise from a variety of exogenous and endogenous insults that, when combined with genetic and lifestyle factors, contribute to overall cancer risk (1). Recently, the Director of the National Cancer Institute, Dr. Andrew von Eschenbach, set the challenge goal to “eliminate the suffering and death from cancer by 2015” (2). Successful attainment of this goal will require the integration of multiple levels of scientific investigation, including clinical and epidemiologic research, behavioral studies, animal studies, and basic molecular and cellular biologic research, to develop new strategies for the prevention and treatment of cancer.

Progress in each of these areas will be essential to this effort,

although in our view animal model studies play a critical central role in accelerating the diffusion of knowledge across the disciplines. Animal models have contributed significantly to our understanding of the carcinogenesis process and ways to interfere with that process over the past century (1). They continue to be important for identifying mechanisms underlying the causes and prevention of cancer and for confirming and refining (under controlled experimental conditions) potential leads from human studies showing associations between certain risk factors (both protective and harmful) and cancer risk. In addition, preclinical studies in animal models of cancer are a critical step in the process of translating basic mechanistic findings from the lab bench to the clinic or community. Our group has been working to develop relevant animal models for cancer prevention research and aims to: 1) characterize the molecular mechanisms underlying effective modulators of cancer risk; 2) capitalize on mechanistic information to develop effective combination regimens; and 3) develop surrogate endpoint biomarkers that can be translated to human studies.

The prevalence of obesity has risen steadily for the past several decades in the U.S. According to recent surveillance data (3), nearly two-thirds of U.S. adults are overweight (defined as having a BMI  $\geq 25$  kg/m<sup>2</sup>), and nearly one-third are obese (defined as having a BMI  $\geq 30$  kg/m<sup>2</sup>). Particularly

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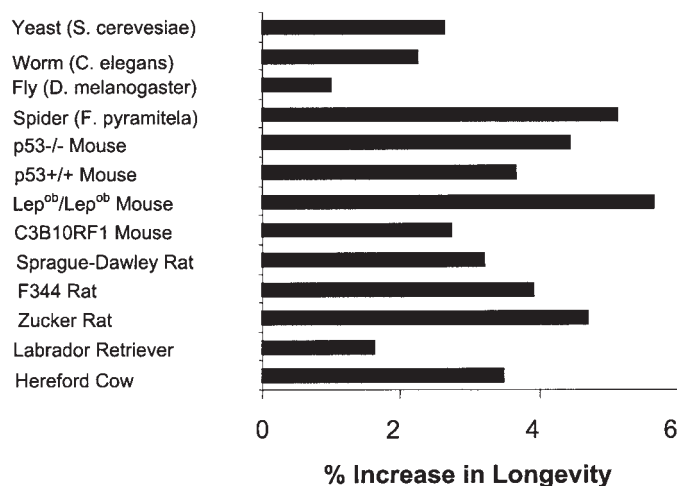
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alarming are the increasing rates of obesity among children and adolescents, portending further increases in the rates of adult obesity and obesity-related cancers. The underlying cause of the obesity epidemic is thought to be a combination of excess caloric intake and insufficient physical activity (4). Calle et al. (5), in a large prospective cohort study of men and women, recently provided strong evidence for a connection between obesity and increased mortality from most forms of cancer. Considering the wide spectrum of cancers associated with obesity in what is, by far, the largest prospective cohort study to date of that connection, these findings suggest an underlying biologic mechanism related to both obesity and carcinogenesis. To date, the relationship between obesity and cancer has not been well studied, and further research on the biologic mechanisms underlying the energy balance-cancer link is urgently needed to fill these gaps in our understanding and facilitate the development of new cancer prevention strategies.

When considering modeling energy balance in cancer models, it is helpful to consider the factors that contribute to an organism's overall energy balance. Energy intake is one part of the equation, and its components include the total amount of energy consumed as well as the source of that energy in the diet and the pattern of food consumption. In contrast, physical activity, growth, energy storage, routine metabolism, and thermoregulation are on the output side of the energy balance equation (6).

In terms of cancer, the energy intake side of the equation has been best studied, particularly comparing ad libitum (unlimited) access to food with calorie restriction (CR)<sup>3</sup> in the range of a 20–40% reduction in caloric intake relative to ad libitum food consumption (7). As shown in **Figure 1**, CR has been shown to increase longevity in multiple species and exert inhibitory effects against a variety of spontaneous neoplasias in several experimental model systems (8–18). CR also suppresses the carcinogenic action of several classes of chemicals in rodents, including polycyclic aromatic hydrocarbons, e.g., benzo(a)pyrene, and dimethylbenz(a)anthracene (DMBA); alkylating and methylating agents, e.g., diethylnitrosamine; and aromatic amines, e.g., *p*-cresidine (19). In addition, CR inhibits several forms of radiation-induced cancers (19). Thus, the inhibitory action of CR on carcinogenesis is effective in several species, for a variety of tumor types, and for both spontaneous tumors and chemically induced neoplasias. Despite the reproducibility and breadth of the beneficial anti-aging and anti-cancer effects of CR, the underlying biological mechanisms of CR are not well understood. Furthermore, the lessons learned from CR research have not yet been effectively translated into strategies for human health promotion or disease prevention.

The recent development of genetically engineered mouse strains with cancer-related genes overexpressed or inactivated provides investigators with powerful tools for studying carcinogenesis and for testing preventive strategies that can offset increased genetic susceptibility to cancer as a result of specific genetic lesions in humans (20). Our work has focused on preventing cancer by dietary interventions, particularly obesity prevention/energy balance modulation, in mice deficient in the p53 tumor suppressor gene, the most frequently altered gene in human cancer. We describe here the results from these studies and discuss future directions for mechanism-based cancer prevention research using relevant animal models.



**FIGURE 1** Legend. Increased longevity associated with calorie restriction (CR) in diverse model species, including p53 knockout mice (refs. 8–18). These studies involve 30–40% CR with 3 exceptions: 1) Yeast were reared on 2% vs. 0.5% Glucose media (Lin et al., 2002); 2) Worm data were obtained by comparing strains with genetic defects in feeding rate with control strains (Lakowski and Hekemi, 2000); 3) Zucker rats were restricted ~18% relative to controls (Johnson et al., 1997).

### p53-Deficient mice

Mutation of the p53 tumor suppressor gene is the most frequently observed genetic lesion in human cancer; over 50% of all human tumors examined to date have identifiable p53 gene point mutations or deletions (21). Donehower et al. (22) first reported in 1992 that homozygous p53-knockout (p53<sup>-/-</sup>) mice are viable but highly susceptible to spontaneous tumorigenesis (particularly lymphomas) at an early age. p53<sup>-/-</sup> mice have been useful tools for studying the role of p53 in carcinogenesis. For example, in response to a DMBA-induced skin carcinogenesis protocol, p53<sup>-/-</sup> mice, relative to wild-type (p53<sup>+/+</sup>) mice, show no difference in benign papilloma formation but display greatly accelerated progression to malignant carcinomas (23). Furthermore, the carcinomas formed in the p53<sup>-/-</sup> mice show higher indices of malignancy as measured by histopathology, further confirming the importance of p53 loss in acceleration of tumor progression. p53-deficient mice also provide an attractive and 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.

### Cancer prevention studies in p53-null (p53<sup>-/-</sup>) mice

We have evaluated the ability of several dietary and chemopreventive interventions to offset the increased susceptibility of p53<sup>-/-</sup> mice to spontaneous tumorigenesis (8,24–26). Given its potency in a variety of models and tissue types, we chose CR as our initial proof of principle that a dietary intervention could influence tumorigenesis in mice predestined to develop tumors due to a lack of the p53 tumor suppressor. In p53<sup>-/-</sup> mice, CR (60% of the control group's energy intake, achieved by reducing carbohydrate calories) increases the latency of spontaneous tumor development (mostly lymphomas) ~75% and significantly slows thymocyte and splenocyte cell cycle traverse (24). The time to tumor onset in these mice is largely p53-dependent, with the major-

<sup>3</sup> Abbreviations used: CR, calorie restriction; IGF-1, insulin-like growth factor-1; p53<sup>-/-</sup>, p53-null; p53<sup>+/-</sup>, heterozygous p53-knockout; p53<sup>+/+</sup>, p53 wild-type; RR, relative risk.

ity of  $p53^{-/-}$  mice developing and dying from spontaneous tumors by approximately 6 months of age compared to nearly 2 years for  $p53^{+/+}$  mice. However, the highly statistically-significant tumor-delaying effect of CR, relative to ad libitum consumption, is similar in both  $p53^{-/-}$  and wild-type ( $p53^{+/+}$ ) mice, indicating the mechanisms underlying CR may be  $p53$ -independent (8).

We have 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 decreases adiposity and significantly delays spontaneous tumorigenesis in  $p53^{-/-}$  mice and, in particular, nearly eliminates lymphoma development (25). Furthermore, the DHEA analogue 16- $\alpha$ -fluoro-5-androsten-17-one (fluasterone; 0.15% in the diet) also decreases adiposity and suppresses spontaneous lymphoma development and lengthens survival in  $p53^{-/-}$  mice (26). The anti-lymphomic effects of these chemopreventive steroids are strongly associated with decreased body weight as well as with delayed thymocyte maturation and increased apoptotic rates of premalignant thymocytes [(26,28,29); Kim, Hursting, and Perkins, unpublished results]. 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, can be offset, at least in part, by preventive approaches.

$p53^{-/-}$  mice have also been useful for elucidating the mechanisms of action underlying the tumor-inhibitory effects of CR and the chemopreventive steroids. For example, the anti-tumor effect of DHEA (or its fluorinated analogue fluasterone) in  $p53^{-/-}$  mice is independent of its effects on quantity of food intake or on nucleotide pool levels (26), as had previously been suggested (27). Wang et al. showed that both CR and DHEA decrease thymocyte proliferative rates (28). Poetschke et al. (29) showed that calorie restriction, DHEA, and fluasterone each slow thymocyte cell cycle progression, partially blocks thymocyte maturation, and induce apoptosis in immature thymocytes, the subpopulation of thymocytes from which lymphomas arise in  $p53^{-/-}$  mice. However, the apoptosis-inducing effects of the chemopreventive steroids appear to be mediated by decreased Bcl-2 gene expression, while the effects of CR on apoptosis are independent of the Bcl-2/ Bax apoptotic regulatory pathway. On the other hand, CR (but not the steroids) significantly reduces circulating IGF-1 levels (30), which as suggested by Dunn et al. (31) may be responsible for the apoptotic-inducing effects of CR. Both CR and the chemopreventive steroids also decrease serum leptin levels (Hursting et al., unpublished results). Leptin, the so-called fat hormone, has been shown to act as a pro-inflammatory cytokine (32), a pro-angiogenic factor (33), and also an apoptotic regulator in certain cell types (34), so this reduction in leptin levels may also contribute to the effects of CR. In addition, Mei et al. showed that CR, DHEA, and fluasterone each suppress nitric oxide levels and down-regulate nitric oxide synthetase expression (35). The roles of IGF-1, leptin and nitric oxide and other inflammatory components in the anti-cancer effects of CR in  $p53^{-/-}$  mice are currently being further characterized.

### Cancer 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 due to decreased  $p53$  gene dosage, such as individuals with Li-Fraumeni Syndrome (36). The spontaneous tumors that most frequently occur in

$p53^{+/-}$  mice (hematopoietic neoplasias and osteosarcomas) are similarly observed in humans with Li-Fraumeni Syndrome. The incidence rates of the 2 most common epithelial tumors observed in Li-Fraumeni patients (lung tumors in males and breast tumors in females) vary depending on the background strain of the  $p53$ -deficient mice (37,38). Tumor latency in  $p53^{+/-}$  mice (median survival  $\sim$ 18 mo) is reduced relative to  $p53^{+/+}$  mice (median survival  $\sim$ 26 mo), although is much longer than for  $p53^{-/-}$  mice (median survival  $\sim$ 6 mo). CR and a one-day per week fast both significantly delay spontaneous tumor development (mostly lymphomas and various sarcomas) in male  $p53^{+/-}$  mice, even when interventions are begun in adulthood (30).

While  $p53^{+/-}$  mice have low rates of spontaneous tumorigenesis for up to 12 mo of age, they do display increased susceptibility to chemically induced tumor development relative to wild-type mice. *p*-Cresidine-induced bladder tumors (31), dimethylnitrosamine-induced liver tumors (37), nitrosomethylurea-induced lymphomas (S. Perkins and S. Hursting, unpublished results), and radiation-induced lymphomas and sarcomas (39) all appear significantly earlier in  $p53^{+/-}$  mice than in similarly-treated  $p53^{+/+}$  mice. As mentioned previously, malignant progression of DMBA-induced skin papillomas also occurs much faster in  $p53^{+/-}$  mice than in  $p53^{+/+}$  mice (23). These findings suggest that  $p53^{+/-}$  mice exhibit increased sensitivity to several classes of mutagenic carcinogens when compared to  $p53^{+/+}$  mice, and appear to be susceptible to at least some low-dose, chronic carcinogen regimens that more closely mimic human exposures.

Using the *p*-cresidine-induced bladder tumor model in male  $p53^{+/-}$  mice, we showed that CR (started after tumors had formed) suppresses bladder tumor progression (31). Furthermore, IGF-1 appears to mediate the CR response, as restoration of serum IGF-1 levels in CR mice via osmotic pump infusion reverses the CR effect. We had previously reported (40) a similar finding of a mediating role for IGF-1 in the anti-cancer effects of CR using a Fischer rat leukemia model. As demonstrated by these studies, genetically engineered mice, such as  $p53$ -deficient mice, have tremendous potential for developing models facilitating the study of gene-environment interactions relevant to human cancer prevention.

### Experimental evidence for the role of IGF-1 in cancer

The possible involvement of IGF-1 in cancer was first observed in *in vitro* studies, which consistently showed that IGF-1 enhances the growth of a variety of cancer cell lines (41). These include prostate, bladder, breast, lung, colon, stomach, esophagus, liver, pancreas, kidney, thyroid, brain, ovarian, and cervical and endometrial cancer cell lines (41–43). IGF-1 acts directly on cells via the IGF-1R, which is overexpressed in many tumors, or indirectly through its action with other cancer-related molecules, including  $p53$ . For example, IGF-1 and the  $p53$  tumor suppressor appear to function together in a regulatory network.  $p53$  regulates the expression of IGFBP-3 (44) and IGF-1-induced mitogenesis is associated with phosphorylation and translocation of the  $p53$  protein from the nucleus to the cytoplasm (45).

A markedly increased average and maximal life span and decreased susceptibility to cancer is also observed in several strains of mutant or genetically modified mice that suffer defects in the production of growth hormone or IGF-1 or in responsiveness to growth hormone (and hence express significantly lower levels of circulating IGF-1). The “little” mouse, which is defective in its response to hypothalamic growth hormone-releasing hormone, lives 20–25% longer than wild-

type mice (46). Laron mice, with a disruption in the growth hormone receptor/binding protein gene, have increased circulating levels of growth hormone but greatly reduced serum IGF-1 levels and also live 38–55% longer than wild-type mice (47). Mice with primary deficiencies in growth hormone, prolactin, and thyrotropin, caused by failure of the pituitary to differentiate during fetal development, live 40 to 64% longer than wild-type mice. These latter examples include the Snell and Jackson dwarf mice, which have a point mutation in the homeotic transcription factor Pit1 (48), and the Ames dwarf mouse, which fails to express Pit1 because of an inactivating point mutation in the Prop1 transcription factor (49). As seen with CR, these mutations appear to reduce the onset and/or rate of aging and age-associated cancers. In contrast, tissue-specific overexpression of IGF-1 via the keratin 5 promoter results in increased spontaneous tumor development (50) and increased susceptibility to carcinogens, including *p*-cresidine (Hursting et al., unpublished results).

### **Epidemiological evidence of a role for IGF-1 in human cancer**

There is an abundance of epidemiological evidence that supports the hypothesis that IGF-1 may be involved in human cancer. In a case-control study nested within the Nurses Health Study cohort, Hankinson et al. (51) found that elevated serum IGF-1 levels are associated with an increased risk of developing breast cancer in premenopausal women [relative risk (RR): 2.3; CI: 1.1–5.2] but not in postmenopausal women. Yu et al. also found associations between IGF-1 levels and premenopausal breast cancer risk in Chinese women (52). In contrast, Kaaks et al. (53) found no association between IGF-1 levels and premenopausal breast cancer risk in Swedish women but suggested a possible association with postmenopausal breast cancer risk. Chan et al. (54) in the Physicians' Health Study cohort found that plasma IGF-1 levels were associated with a higher risk of developing a prostate cancer (RR: 4.3; CI: 1.8–10.6). Yu et al. (55) reported that IGF-1, but not IGF-2 or IGFBP-3 levels, was associated with lung cancer. Ma et al. (56) found that both elevated levels of IGF-1 and decreased levels of IGFBP-3 were associated with an increased risk of developing colon cancer in men in the Physicians' Health Study. High plasma levels of IGF-1 and low levels of IGF binding protein-3 have been associated with an increased risk of bladder cancer (57), while reduced risk of childhood leukemia in association with higher IGFBP-3 levels has also been reported (58). The epidemiological association between IGF-1 and IGFBP levels and the risk of various cancers certainly requires more investigation, including additional prospective studies to better establish the temporal nature of any associations. However, when considered together, the multiple human studies reported to date suggest that components of the IGF-1 system are risk factors important in the development of several human cancers.

### **Summary and future directions**

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 genetically engineered mice, gene expression microarrays, and proteomics to identify additional modulatable targets and make important progress towards one of the major goals in

contemporary cancer research: the development of effective mechanism-based strategies for preventing human cancer. In this review, examples of cancer prevention studies that have utilized p53-deficient mouse models were discussed. 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. Further development of relevant animal models for prevention studies and the incorporation of new technologies (such as microarrays and proteomics) into these studies are approaches that we are taking to accelerate the pace of mechanism-based cancer prevention research. For example, to further study diet-p53 interactions in mammary carcinogenesis, we have been characterizing a rapid and spontaneous p53-deficient mouse mammary tumor model developed by crossing p53<sup>+/-</sup> mice with MMTV-*Wnt-1* transgenic mice (59). In these mice CR, a one day/wk fast, the synthetic retinoid fenretinide, tamoxifen, and the chemopreventive steroid flasterone each delay spontaneous mammary tumor development (Hursting et al., unpublished results). Furthermore, p53 gene dosage impacts the magnitude of these preventive effects.

Regarding studies of energy balance and cancer, which are essential given the impact of obesity on cancer development and the paucity of mechanistic data on this association, we are in the process of comparing and combining CR and exercise in our p53-deficient mouse models, as well as other tumor models. This includes APC<sup>min</sup> mice, which spontaneously develop preneoplastic intestinal polyps that can be suppressed by CR and exercise (60,61). We are also further investigating the role of IGF-1, other hormones, and body composition in the energy balance and cancer relationship. For example, to elucidate the molecular response underlying the anticancer effects of CR, and to determine which of the CR-responsive genes are IGF-1-dependent, we are using a strategy employing oligonucleotide microarrays. Preliminary analyses of hepatic RNA from a 4-wk study in wild-type (C57 BL/6) mice fed ad libitum or 20, 30, or 40% CR and receiving either a placebo pellet or an IGF-1 pellet implanted subcutaneously suggest that CR induces changes in the expression of multiple genes associated with phase 1 and phase 2 xenobiotic metabolism, steroid hormone metabolism, cell cycle/DNA repair, and the IGF-1 pathway. Our approach will continue to include the development and use of relevant animal models as well as the adoption of new technologies to accelerate the discovery and characterization of effective cancer preventive interventions and their translation to human populations.

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