Effects of Energy Balance on Cancer in Genetically Altered Mice1

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ABSTRACT Evidence has accumulated from laboratory-based animal experiments and population-based human epidemiological studies that lifestyle factors that affect energy balance, such as caloric intake, nutritional status, and exercise, act in concert with genetic susceptibility to influence cancer development and progression. The use of animal models with specific genetic alterations, in combination with lifestyle modifications that alter overall energy balance, has contributed to a greater understanding of the mechanistic changes occurring during carcinogenesis and to the identification of points of intervention. Studies in our laboratory focusing on the role of energy balance and genetic susceptibility in mice deficient in one (+/−) or both (−/−) alleles of the p53 tumor suppressor gene and mice with a mutant APC allele (APCMin) showed that calorie restriction decreases tumor burden, increases tumor latency, and decreases serum insulin-like growth factor (IGF)-1 and leptin levels. Data from our studies, combined with results from other animal and human studies, have established a role for IGF-1 in carcinogenesis. Studies using genetic models of cancer that have been interbred with mice with abnormal levels of IGF-1 will enable the examination of combined effects of energy balance and genetic alterations on the cancer process. Models that integrate lifestyle and genetic effects in a single system provide a physiologically intact system in which combination interventions and therapies for cancer prevention can be tested and validated, thus building a strong preclinical foundation that will inform the development of clinical trials and add perspective to epidemiological studies. J. Nutr. 134: 3394S–3398S, 2004.

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

Carcinogenesis is a multistage process during which individual cancer cells undergo a series of genetic and epigenetic alterations (1). Understanding the lifestyle factors that affect energy balance in combination with genetic, behavioral, and environmental factors that contribute to this disease process is central to developing cancer prevention strategies and interventions that will lighten disease burden and will contribute to increased survival. Mouse models are in a unique position to enable the delineation of the various factors involved in cancer development by offering an experimental approach that can test preventive strategies and can offset genetic susceptibilities (2,3).

Our approach has been to understand the effect of lifestyle modifications related to alterations in energy balance on the physiology of normal mice and also in genetic and transgenic models of human cancer. We use this information to evaluate the effect of nutritional interventions on energy balance, genetic susceptibility and, in turn, cancer development. The goals of our research in the area of cancer prevention have been to 1) identify molecular mechanisms and pathways that contribute to cancer development and risk in multiple models, 2) use the information on the molecular mechanisms and pathways to create evidence-based combination interventions and therapies, and 3) identify novel cancer biomarkers for mouse and human studies. This review will highlight studies from our laboratory as well as others that have investigated the role of underlying mechanisms of energy balance and genetic susceptibility in cancer prevention. Our aim is to prompt readers to actively think about how the interaction between modifiable lifestyle factors and genetics influences the design and outcome of their studies.

Energy balance and lifestyle factors

Energy balance, referring to a balance between caloric intake and expenditure, has received growing attention in the lay and scientific communities, given the role of overnutrition

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in the increasing trends in obesity in the United States (4,5). Traditional studies on energy balance have emphasized energy expenditure as the main culprit resulting in imbalance (6). However, recent studies using more sophisticated techniques, such as doubly labeled water, to measure total energy expenditure indicate that total energy intake with respect to undernutrition and overnutrition plays a much greater role in energy imbalance than previously thought (6).

Recent evidence from a large prospective cohort study provides strong supporting evidence in both men and women for the link between obesity and increased mortality from most cancer types (7). Although the increased incidence of obesity is believed to be related to excess caloric intake and reduced physical activity, the biological relationship between cancer and obesity is not well established (8). Animal studies that model energy balance by taking into consideration energy intake and expenditure are central to identifying the biological basis of the energy-balance cancer link and to developing prevention strategies (9). More specifically, energy intake incorporates total energy consumption, energy source, and food consumption patterns, whereas energy expenditure incorporates physical activity, growth, energy storage, routine metabolism, and thermoregulation (9). Related to energy balance are modifiable lifestyle factors, such as calorie intake, physical activity, and diet and their relationship to cancer.

To date calorie restriction (CR) studies in animals best address the relationship between energy intake and cancer outcomes. CR dietary regimens limit total energy intake by 20–40% in comparison with free-access food consumption, while providing essential nutrients and vitamins, without causing malnutrition (10). CR increases longevity in multiple species and slows several age-related biological functions, including immune function and tumorigenesis (11–21). CR also inhibits the carcinogenic potential of chemical- and radiation-induced cancers (22). The underlying biological mechanisms of CR are not well understood despite the reproducibility and breadth of the beneficial antiaging and anticancer effects of CR. The advent of mouse models with specific genetic lesions has made it possible to examine the ability to offset increased cancer risk due to genetic lesions, such as loss of p53, using preventive strategies, such as CR.

Cancer prevention studies in p53−/− and p53-heterozygote mice

Aberrations in the p53 tumor suppressor gene have been documented in over 50% of all human tumors, making it the most frequently observed genetic cancer lesion (23). Mice deficient in both p53 alleles [p53 null (p53−/−)] are highly susceptible to spontaneous tumorigenesis, namely lymphomas, whereas mice heterozygous for p53 (p53+/−) display elevated rates of spontaneous tumors (hematopoietic neoplasias and osteosarcomas) after age 12 mo and display similarities to humans who have increased susceptibility to cancer because of decreases in p53 gene dosage (24,25). Because CR can positively affect carcinogenesis in a variety of models and tissue types, it was selected as an initial dietary intervention in our studies with the p53−/− and p53+/− models. In p53−/− mice, 40% CR (60% of the control group’s energy intake, achieved by reducing carbohydrate calories) increased the latency of spontaneous tumor development (mostly lymphomas) and significantly reduced thymocyte and splenocyte cell cycle traverse (26). Similarly, CR and a fast of 1 d/wk significantly delays the development of spontaneous tumors (mostly lymphomas and various sarcomas) in p53+/− mice (27). The highly statistically significant tumor-delaying effect of CR, relative to free-access feeding, is similar in both p53−/− and p53 wild type (p53+/+) mice, indicating that the preventive effects of CR are independent of the functional status of p53 (11). In addition, the increased susceptibility of the p53+/− mice to p-cresidine–induced bladder tumors is reduced in mice on a CR regimen (24). Evidence suggests that IGF-1 modulates the CR effect because restoration of IGF-1 via an osmotic pump in this model negates the effect of CR on tumor progression (24,28).

Furthermore, the chemopreventive steroids dehydroepiandrosterone (DHEA; 0.3% in the diet) and the DHEA analogue 16α-fluoro-5-androsten-17-one (fluasterone; 0.15% in the diet) significantly delayed spontaneous tumorigenesis in the p53−/− mice (29,30). Taken together with the effect of CR in the p53−/− mice, these studies are useful in understanding the mechanisms through which tumor progression is delayed. The p53−/− mice have also been useful for elucidating the mechanisms of action underlying the tumor-inhibitory effects of CR and the chemopreventive steroids. These studies support previous results indicating that the antitumor effect of DHEA and fluasterone is independent of its effects on quantity of food intake or on nucleotide pool levels (30,31). Additionally, CR, DHEA, and fluasterone slow thymocyte cell-cycle progression, partially block thymocyte maturation, and induce apoptosis in immature thymocytes, the subpopulation of thymocytes from which lymphomas arise in p53−/− mice, but through seemingly different pathways (9,32). The observed apoptosis-inducing effects of the chemopreventive steroids are mediated by decreased Bcl-2 gene expression, whereas CR effects on apoptosis are independent of the Bcl-2/Bax apoptotic regulatory pathway (31,33). Conversely, CR, but not the steroids, significantly reduces circulating IGF-1 levels (27), which [as suggested previously (24)] may be responsible for the apoptotic-inducing effects of CR. Both CR (27) and the chemopreventive steroids also decrease serum leptin levels [S. N. Perkins and S. D. Hursting, National Cancer Institute (NCI), 2004, unpublished results]. Leptin, commonly referred to as the fat hormone, has been shown to act as a proinflammatory cytokine (34), a proangiogenic factor (35), and an apoptotic regulator in certain cell types (36). This observed reduction in serum leptin levels may also contribute to the effects of CR. Furthermore, CR, DHEA, and fluasterone suppress nitric oxide levels and downregulate nitric oxide synthetase expression (37). With these findings, a more complex picture begins to evolve regarding the different roles for IGF-1, leptin, nitric oxide, and other inflammatory components in the anticancer effects of CR. Both of the p53−/− and the p53+/− models in combination with dietary, nutritional, and chemopreventive agents have provided greater insight into the interaction between genetics and the effect of lifestyle interventions, namely CR, in the carcinogenesis process.

Cancer prevention studies in mice with altered IGF-1 levels

Evidence for the role of IGF-1 in cancer first came from in vitro studies of various cell lines, including prostate, bladder, breast, lung, colon, stomach, esophagus, liver, pancreas, kidney, thyroid, brain, ovarian, cervical, and endometrial, in
which IGF-1 enhanced cell proliferation (38). IGF-1 promotes cell growth either directly by binding the IGF-1 receptor on cells or indirectly through its interaction with other cancer-related molecules, including p53 (39). The p53 regulates the expression of IGF binding protein (IGFBP)-3, which binds circulating IGF-1, and, in turn, IGF-1–induced mitogenesis is associated with phosphorylation and translocation of the p53 protein from the nucleus to the cytoplasm (39,40).

Epidemiological evidence strongly supports the involvement of IGF-1 in human cancer. A nested case–control study within the Nurses Health Study cohort found that elevated serum IGF-1 levels are associated with an increased risk of developing breast cancer in premenopausal women (relative risk: 2.3; CI: 1.1–5.2) but not in postmenopausal women (41). Similar associations were found between IGF-1 levels and premenopausal breast cancer risk in Chinese women (42). In contrast, a study in Swedish women found no association between IGF-1 levels and premenopausal breast cancer risk but suggested a possible association with postmenopausal breast cancer risk (43). In the Physician’s Health Study cohort, IGF-1 levels were associated with higher risks of developing prostate and colon cancer (44,45). In other studies, high plasma levels of IGF-1 and low levels of IGFBP-3 are associated with an increased risk of bladder cancer (46), whereas a reduced risk of childhood leukemia is associated with higher IGFBP-3 levels (47). The numerous human studies reported to date collectively suggest that components of the IGF-1 system are risk factors important in the development of several human cancers, although the further understanding of the association between IGF-1 and IGFBPs in epidemiological studies requires additional investigation.

Thus, given the role of IGF-1 in human studies and various mouse models, including the aforementioned p53+/− and p53+/− CR mice, studies involving mice with genetically altered levels of IGF-1 merit further study. The bovine keratin 5 promoter IGF-1 transgenic (BK5.IGF-1) mice and the liver IGF-1 deficient mice (LID), which express increased and decreased levels of IGF-1, respectively, present interesting models in which to examine the effects IGF-1 and energy balance in cancer prevention (48,49). Tissue-specific overexpression of IGF-1 in the BK5.IGF-1 model contributes to increased spontaneous tumor development and increased susceptibility to carcinogens, including β-cresidine, that can be offset by CR (48; S. D. Hursting, NCI; J. A. Lavigne, NCI; L. Beltran, University of Texas MD Anderson Cancer Center; D. C. Haines, SAIC-Frederick; S. N. Perkins, NCI; H. H. Wimbrow, SAIC-Frederick; L. O. Baum, Baylor College of Medicine; W. G. Alvord, Data Management Services, NCI-Frederick; J. C. Barrett, NCI; J. DiGiovanni, University of Texas MD Anderson Cancer Center; 2004, unpublished results).

An increase in the average and the maximal life span and decreased susceptibility to cancer is 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, which result in decreased levels of circulating IGF-1. For example, the “little” mice, which are defective in their response to hypothalamic growth hormone releasing hormone, live 20–25% longer than wild-type mice (50), and Laron mice, which have 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 live 38–55% longer than wild-type mice (51). Additionally, the Snell and Jackson dwarf mice, which have a point mutation in the homeotic transcription factor, Pit1, and the Ames dwarf mouse, which fails to express Pit1 because of an inactivating point mutation in the Prop1 transcription factor, have primary deficiencies in growth hormone, prolactin, and thyrotropin, and live 40–64% longer than wild-type mice (51,52). Similar to CR and subsequent reduction in IGF-1 levels, these mutations appear to reduce the onset, rate, or both, of aging and age-associated cancers.

Studies in LID mice provide a strong link between reduction in IGF-1 levels and delayed tumor onset. The LID mice were created using the Cre/loxP recombination system to delete the igf1 gene in the liver (49). In contrast to the igf1 gene knockout mice, which develop severe developmental disorders, the LID mice were normal with respect to growth and development despite a 75% reduction in serum IGF-1 levels (49).

This reduction in serum IGF-1 levels in LID mice mimics the reduction in circulating IGF-1 observed with CR and thus provides a model for investigating the mechanistic relationship between energy balance and genetic background on tumor development. To investigate the effects of decreased circulating IGF-1 on colon cancer growth and metastasis, Wu et al. (53) orthotopically transplanted colon 38 adenocarcinoma tissue fragments onto the cecum of LID and wild-type mice. The LID mice displayed both a decreased incidence of tumor growth on the cecum and fewer liver metastases than did wild-type littersmates. Additionally, LID mice either exposed to the mammary-specific chemical carcinogen dimethylbenz(a)anthracene or crossed with mammary tumor prone C3(1)/SV40 large T-antigen transgenic mice displayed 26–30% difference in mammary tumor development compared with control mice with normal IGF-1 levels (54). On the basis of these results, it would be informative to compare the tumor development in LID and CR mice to determine whether CR effects are mediated through pathways other than the IGF-1 pathway.

Cancer prevention studies in the ApcMin model

Extensive research into the mechanisms and therapeutic interventions for colorectal cancer has focused on the ApcMin mice, which spontaneously develop neoplastic intestinal polyps because of a fully penetrant dominant mutation converting codon 850 of the murine APC gene from a leucine to a stop codon (55–57). Given that mutation of the APC gene is common to most human colon cancers, the ApcMin mouse, despite its differences in polypl location compared with the human disease, is an excellent example of the utility of animal models in understanding the connection between energy balance and genetic susceptibility to cancer and in developing combination interventions (56).

The preneoplastic lesions in ApcMin mice were shown to be suppressed by modifiable lifestyle factors and chemopreventive agents. Intestinal polyp burden was significantly reduced in ApcMin mice after both a 4-wk and a 10-wk 40% CR regimen (58; A. C. Patel, NCI; H. E. Zeytin, NCI; V. Mai, University of Maryland School of Medicine; S. N. Perkins, NCI; S. D. Hursting, NCI; J. W. Greiner, NCI; 2004, unpublished results). IGF-1 levels, along with inflammatory markers, were reduced in mice fed the CR diet (58). Additionally, mice fed an olive-oil–based diet high in fruits and vegetables reduced overall polyp numbers in the 10-wk intervention, suggesting a role for dietary-based anti-inflammatory compounds in cancer prevention (58). Colbert et al. (59) showed that involuntary exercise via a treadmill demonstrated no overall effect on polyp burden in ApcMin mice. However, when female and male mice were analyzed separately, involuntary exercise resulted in fewer colon and total polyps in male mice. Current studies in our laboratory indicate that voluntary running wheel
exercising significantly affects polyp burden (L. H. Colbert, University of Wisconsin; V. Mai, University of Maryland School of Medicine; S. N. Perkins, NCI; S. D. Hursting, NCI; 2004, unpublished results). Studies are underway to determine commonalities and differences in the mechanisms mediating decreased tumor burden in the presence of multiple modifiable lifestyle factors, with a focus on the role of the innate and adaptive immune system.

A prime example of the efficacy of combination therapies involves the administration of both celecoxib and a carcino-embryonic antigen (CEA) based vaccine in CEA.Tg/ApcMin mice (60). Most of the mice receiving the combination intervention developed no polyps, resulting in better health status and increased survival (60). Understanding the molecular mechanism leading to this dramatic decrease in polyp burden will be key to developing novel intervention strategies that take into account energy balance. This combination regimen is of particular relevance in high-risk individuals, such as those with the genetic syndromes of familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer. Treatment with celecoxib reduced polyp numbers in most familial adenomatous polyposis patients over a 6-mo period (61). The potential to combine celecoxib treatment with a cancer vaccine in this high-risk population, based on the results from preclinical animal, is nothing short of exciting.

Summary and future directions

The potential to delay genetically driven tumor development with modifiable lifestyle factors and chemoinmunopreventive agents, thereby leading to the identification of underlying molecular mechanisms, presents an evidence-based approach to cancer prevention. The growing sophistication of animal models enables cancer researchers to test the applicability of interventions in physiologically relevant animal models that incorporate both genetic susceptibility to disease and energy balance. As we attempt to tackle cancer in the twenty-first century, we are armed with an arsenal of tools—such as genetically engineered mice, gene expression microarrays, and the interplay among lifestyle factors, genetic susceptibility, and the immune response.

The days of altering a single gene and figuring out its role in tumorigenesis are gone. We are now in a transition period of modifying the animal models that exist and gathering information from the literature on how to adapt these models to reflect the influence of lifestyle modifications and how to design combination approaches to cancer prevention. Research in cancer prevention demands the integration of biological, epidemiological, and behavioral disciplines to identify efficacious and applicable interventions that will drive cancer prevention in the human population.

LITERATURE CITED