Increased Tumor Growth in Mice with Diet-Induced Obesity: Impact of Ovarian Hormones

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Obesity increases the risk of many cancers in both males and females. This study describes a link between obesity, obesity-associated metabolic alterations, and the risk of developing cancer in male and female mice. The goal of this study was to evaluate the relationship between gender and obesity and to determine the role of estrogen status in obese females and its effect on tumor growth. We examined the susceptibility of C57Bl/6 mice to diet-induced obesity, insulin resistance/glucose intolerance, and tumors. Mice were injected sc with one of two tumorigenic cell lines, Lewis lung carcinoma, or mouse colon 38-adenocarcinoma. Results show that tumor growth rate was increased in obese mice vs. control mice irrespective of the tumor cell type. To investigate the effect of estrogen status on tumor development in obese females, we compared metabolic parameters and tumor growth in ovariectomized (ovx) and intact obese female mice. Obese ovx female mice developed insulin resistance and glucose intolerance similar to that observed in obese males. Our results demonstrate that body adiposity increased in ovx females irrespective of the diet administered and that tumor growth correlated positively with body adiposity. Overall, these data point to more rapid tumor growth in obese mice and suggest that endogenous sex steroids, together with diet, affect adiposity, insulin sensitivity, and tumor growth in female mice. (Endocrinology 147: 5826–5834, 2006)

The prevalence of overweight and obesity has increased dramatically in the United States. Among adults aged 20 yr and older in 2002, approximately 65% were overweight, of which 30% were obese and 5% were extremely obese (1). Epidemiological studies show that obesity increases the risk of numerous cancers in both males and females. Excess body weight is responsible for an estimated 14% of all cancer deaths in men and up to 20% of all cancer deaths in women (2). However, there is a gap in the literature in linking obesity, metabolic alterations associated with obesity, and the risk of developing cancer in males and females. Insights into the mechanism(s) through which obesity increases cancer risk in males and females are urgently needed to develop new strategies for preventing and treating obesity-related cancers.

High-fat/high-calorie diets are associated with increased colorectal cancer risk in epidemiological studies (3–5). Studies with mice fed a high-calorie diet show a significant increase in cellular proliferation in epithelial cells of the pancreas (6), prostate (6), and colon (7, 8). In contrast, a large number of human and animal studies demonstrate that restricted intake of calories (calorie restriction) strongly inhibits carcinogenesis and slows tumor growth (9–13). Calorie restriction significantly decreases serum insulin and IGF-I, which have been linked to carcinogenesis. For example, when IGF-I is given to calorie-restricted p53-deficient mice by infusion, the anticarcinogenic effect of calorie restriction is abolished (14). Similar results were observed when GH or IGF-I was administered to calorie-restricted Fischer rats with mononuclear cell leukemia (15). Additionally, Fischer rats that were injected with azoxymethane (carcinogen that induces polyp formation in the colon with subsequent development of cancer) and treated with insulin had an increased incidence and accelerated growth of colorectal cancer, whereas rats on a high-fat/high-calorie diet were relatively insulin resistant and had impaired glucose tolerance, dyslipidemia, and a higher incidence of aberrant crypt foci in the colon (16). These data suggest that factors that modulate serum insulin and IGF-I such as energy intake, and genetically determined or diet-induced obesity, have a significant effect on cancer risk. The mechanisms underlying this effect are still unknown, but it is possible that oxidative stress, inflammatory cytokines, or other factors are also required to mediate the anticarcinogenic effect of calorie restriction (17, 18).
Postmenopausal women, as well as rodents after ovariectomy, often become obese, suggesting the important role of estrogens in the maintenance of body composition and lipid homeostasis. Ovariectomy-induced obesity in mice is associated with decreased oxygen consumption accompanied by decreased expression of energy expenditure-related genes in adipose tissue and skeletal muscle (19). Estrogen receptor α knockout mice exhibit adipocyte hyperplasia and hypertrophy, insulin resistance, and glucose intolerance in both sexes, suggesting that estrogen signaling is critical in white adipose tissue and is involved in the regulation of energy expenditure (20). Similarly, aromatase knockout mice, which cannot synthesize endogenous estrogens, also exhibit more intraabdominal adipose tissue than their wild-type littermates. This was associated with reduced spontaneous physical activity levels, diminished glucose oxidation, and decreased lean body mass (21).

This study investigates the relationship between diet-induced obesity and gender, the impact of obesity on tumor growth, and the role of estrogen status in obese females. Male and female mice with different degrees of adiposity were obtained by manipulating their caloric intake. After chronic exposure to different caloric regimens, animals were challenged with sc injection of H-59-C10 or MC38 murine tumor cells, and xenograft tumor growth was then monitored. Results of this study provide strong evidence that tumor size increases significantly with increasing adiposity. Removal of endogenous estrogens by ovariectomy in female mice increased their weight, induced insulin resistance, and accelerated tumor growth rate. These findings suggest that endogenous estrogens protect female mice from becoming obese and glucose intolerant and from developing certain types of tumors.

Materials and Methods

Animals and diets

Mice with a lean or obese phenotype were generated by manipulating their caloric intake. For this purpose, mice were either calorie restricted or given free access to a control or high-calorie diet. In the first study, both female and male mice (mixed background of C57BL/6, FVB/N and sv129) were randomized to receive the control diet (22.9% protein, 51% carbohydrate, and 16.1% fat) or the high-calorie diet (20% protein, 36.3% carbohydrate, and 5.4% fat) or the high-calorie diet (20% protein, 36.3% carbohydrate, and 5.4% fat). Mice were randomized to receive the control diet (22.9% protein, 51% carbohydrate, and 5.4% fat) or the high-calorie diet (20% protein, 36.3% carbohydrate, and 5.4% fat).

To determine the effect of sex steroids on obesity and tumor growth, intact and ovariectomized (ovx) mice were used. Ovariectomy (surgical removal of the ovaries) is a well-characterized approach to mimic the postmenopausal state in sexually mature mice. After surgery, estrogens are no longer produced by the ovaries and are not under feedback regulation by gonadotropins (22, 23). Therefore, in the second study, 6-wk-old C57/BL6Ncr ovx or non-ovx female mice (12 per group; Charles River Laboratories, National Cancer Institute, Frederick, MD) were randomized into the following three groups: 1) control diet (22.9% protein, 51% carbohydrate, and 5.4% fat) or the high-calorie diet (20% protein, 36.3% carbohydrate, and 35.5% fat).

To determine the kinase activity of Akt and ERK in response to insulin, IGF-1, and β-estradiol (E2) treatment to determine which of these factors may be responsible for the growth of the cancer cells used in the present studies. Protein extraction and SDS-PAGE were performed as previously described (27). The antibodies used include phospho-AKT/1 (Cell Signaling Technology, Danvers, MA) phospho-Akt, Akt, ERK2 (Santa Cruz Biotechnology, Santa Cruz, CA) and actin (Sigma, St. Louis, MO). Immune complexes were detected using horseradish peroxidase conjugated secondary antisera (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) and enhanced chemiluminescence. Blots were analyzed by densitometry and quantified with MacBas version 2.52 software (Fuji Photo Film).

Cell migration assay

To determine the invasiveness of the cancer cells in response to exposure to the serum from lean and obese mice, we measured the cells’ migration ability. Briefly, 5 × 10^5 H-59-C10 green fluorescent protein-transfected cancer cells were added to the top wells of migration chambers in serum-free DMEM containing 0.2% BSA. The top chambers were placed in 24-well plates and incubated for 24 h at 37 °C and the lower chambers were filled with 5% sera obtained from obese mice or from control mice. After removal of the cells from the upper surface of the filters, the remaining cells on the lower surface were counted randomly in 10 different fields using a fluorescent microscope.

Cancer cells used in animal studies

MC38-colon adenocarcinoma cancer cells were obtained from the Laboratory of Dr. Lee Helman (National Cancer Institute). H-59 Lewis Lung carcinoma cells were previously described (24, 25). In some experiments, H-59 cells were transfected with a retroviral vector expressing green fluorescent protein (they are referred as H-59-C10) (26). Cells lines were maintained in DMEM (BioSource, Camarillo, CA) supplemented with 10% fetal calf serum (Invitrogen, Carlsbad, CA) and gluta- mine (BioSource).

Tumor challenge

To determine the effects of obesity on tumor growth, mice were injected sc with 0.5 × 10^6 tumor cells and monitored daily to check for the presence of palpable tumors. Once tumors became palpable, tumor volume was calculated by measuring the length, width and depth of the tumor with calipers.

Serum hormones

Serum insulin was measured using a rat insulin RIA kit (Linco Research Inc., St. Charles, MO). Leptin was analyzed using direct RIA (Linco Research). IGF-I was measured using a rat RIA kit (Diagnostic Systems Laboratories, Inc., Webster, TX).

Insulin and glucose tolerance tests

To determine the effects of obesity on glucose regulation and insulin sensitivity, we performed the insulin-tolerance test and glucose-tolerance test on our animals. The insulin-tolerance test was performed at noon by ip injection of 0.75 U/kg insulin. The ip glucose-tolerance test was performed after overnight fasting by administering 20% glucose (2 g/kg) to mice. Blood glucose was measured using a Glucometer Elite (Bayer, Elkhart, IN) at the indicated time points.

Body fat content

To compare the adiposity levels of the mice in the various experimental groups, body fat mass was measured using a Bruker minispec NMR analyzer mq 10 in nonanesthetized mice (Bruker Optics, Woodlands, TX).

Protein extraction and Western blot analysis

We examined the kinase activity of Akt and ERK in response to insulin, IGF-1, and β-estradiol (E2) treatment to determine which of these factors may be responsible for the growth of the cancer cells used in the present studies. Protein extraction and SDS-PAGE were performed as previously described (27). The antibodies used include phospho-ERK1/2 (Cell Signaling Technology, Danvers, MA) phospho-Akt, Akt, ERK2 (Santa Cruz Biotechnology, Santa Cruz, CA) and actin (Sigma, St. Louis, MO). Immune complexes were detected using horseradish peroxidase conjugated secondary antisera (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) and enhanced chemiluminescence. Blots were analyzed by densitometry and quantified with MacBas version 2.52 software (Fuji Photo Film).
Colony formation assay

The ability of H-59-C10 cancer cells to form tumors in vitro in response to exposure to the serum from obese and control mice was tested. Colony formation in soft agar was analyzed as described previously (28). Briefly, H-59-C10 cancer cells were cultured in semi-solid agar for 12 d in the presence of 5% sera obtained from obese mice or from mice fed the control diet. Colonies exceeding 250 μm in diameter were scored. The results of two independent experiments performed with sera from female and male mice fed high-calorie and control diets are demonstrated.

Statistical analyses

Values are presented as mean ± se. Statistical significance was determined by one- or two-way ANOVA or t test using SigmaStat software (SPSS Inc., Chicago, IL). Means ± sem are indicated. P < 0.05 was considered statistically significant.

Results

Metabolic changes in obese male and female mice and their effects on tumor growth

Male and female mice were maintained on control or high-calorie diets for 10–14 wk. Figure 1A shows that male and female mice consuming the high-calorie diet for 10 wk gained significantly more body weight than mice on the control diet. The increase in body weight correlated with a 3-fold increase in body fat (Fig. 1B) and 4- to 5-fold increase in serum leptin levels (Fig. 1C). Serum IGF-I levels did not differ significantly between male and female mice fed a control or a high-calorie diet (Fig. 1D). However, white adipose tissue is a significant source of IGF-I and therefore, in obesity state, local IGF-I bioactivity may play a role. Histological examination of livers from obese male mice revealed a significant hepatic lipid accumulation. A similar but less pronounced effect was observed in livers of obese female mice (Fig. 1E).

Several metabolic parameters were also altered in obese mice. Obese male mice had higher serum glucose and 7-fold higher serum insulin than control males (Fig. 2, A and B). Additionally, these mice were insulin resistant and glucose intolerant (Fig. 2, C and D). In contrast, serum glucose and insulin did not differ significantly between obese and control female mice (Fig. 2, A and B). Obese female mice had normal insulin sensitivity but impaired glucose tolerance, especially during the first 60 min after receiving glucose by ip injection (Fig. 2, C and D).

To test whether obesity coupled with insulin resistance and glucose intolerance is also associated with tumor cell growth, tumor growth rates were compared between obese and control mice. Tumors were induced by sc injection of MC38 adenocarcinoma cells or H-59-C10 Lewis lung carcinoma cells (0.5 × 10⁶ cells per injection). Tumor cells were injected sc, and metastases were not detected during the experimental time frame (2 wk). Injected animals were maintained on control or high-calorie diets for 10 wk, and tumor size was monitored for 2 wk after injection. Our results demonstrate that diet-induced obesity increased tumor prevalence (Fig. 3A) and tumor growth (Fig. 3B) in both males and females. This observation was evident in three mouse cohorts and was independent of the xenograft tumor model (Fig 3C).

Of note, in obese animals there was a significant invasion of tumor cells into white adipose tissue (Fig. 3D), and fat deposits were also observed at the periphery and in the center.

Fig. 1. Diet-induced obesity in female and male mice fed a high-calorie (HC) diet. A, Body weights of males and females maintained on control (CD) (n = 27, n = 29, respectively) or HC diet (n = 27, n = 29, respectively) for 10 wk. B, Body fat content as assessed by NMR in males and females maintained on CD or HC diets for 10 wk. C, Serum leptin levels of males and females maintained on CD or HC diets. D, Serum IGF-I levels of males and females fed CD or HC diet. E, Increased lipid accumulation in livers of mice fed HC diet as assessed by hematoxylin and eosin staining of liver sections (*, P < 0.05).
of tumor tissue (Fig. 3E). Taken together, these data suggest that obesity contributes to enhanced tumor development.

Impact of endogenous estrogens on obesity, metabolic parameters, and tumor growth

The aforementioned data indicate that a high-calorie diet results in different metabolic effects in male and female mice. Sex hormones that modulate metabolic changes induced by dietary factors can account for this discrepancy. Indeed, published data support a hypothalamic role of estrogens in mediating food intake and body weight. This compelled us to investigate the role of estrogens in the pathogenesis of diet-induced obesity and to determine how this affects tumor growth. We examined the impact of different diets on ovx and non-ovx female mice. An additional group of mice that was calorie restricted was also tested because several studies...
revealed inhibition of tumor growth in response to calorie restriction (9–11, 13, 29). The presented data (Fig. 4A) demonstrate the efficiency of the ovx procedure because ovx females do not exhibit any estrous cycles. Figure 4, B and C shows that ovx females gained more body weight and body fat at 10 wk than non-ovx females on each diet regimen. Obese non-ovx females had approximately 2-fold higher body fat content than control mice. In contrast, lean calorie-restricted female mice had 2-fold less body fat compared with control females (Fig. 4C). Similar observations were made in ovx females, although ovx mice had higher fat mass on each dietary regimen than non-ovx animals (Fig. 4C). Serum leptin values correlate with percent adiposity after 10 and 20 wk on the different diets (Fig. 4D). Serum IGF-I levels also increased in obese mice and correlated strongly with body adiposity (Fig. 4E). Ovariectomy by itself caused an increase in serum IGF-I levels (Fig. 4E), as has been reported previously in rats (27–31). However, the mechanism of this increase remains unclear. Surprisingly, in calorie restricted non-ovx females, serum IGF-I levels were not decreased significantly compared with females fed the control diet. In contrast, calorie-restricted ovx females had significantly lower serum IGF-I levels than ovx mice fed the control diet.

Insulin sensitivity and glucose tolerance were also studied in ovx and non-ovx female mice. Females fed a calorie-restricted diet were glucose tolerant regardless of ovarian status (Fig. 5, A and B). Ovariectomy had a profound effect on insulin sensitivity and glucose tolerance. In ovx females on the control diet, glucose tolerance was significantly impaired compared with sham-operated control females, which were fed the same diet (Fig. 5B). Both non-ovx and ovx obese females demonstrated severe glucose intolerance requiring more than 120 min for blood glucose to return to the basal level after the glucose challenge (Fig. 5, A and B). Glucose tolerance test reflects not only insulin sensitivity but also β-cell function. The ovx females fed a high-calorie diet demonstrated an extreme insulin resistance, which might involve failure of β-cell function. The insulin tolerance test, which reflects whole body insulin sensitivity, was similar in obese and control ovx females, as seen in our previous female cohort presented in Fig. 2B. However, only ovx females fed the high-calorie diet had an impaired insulin tolerance, which was evident at 60 min after insulin injection (Fig 5C). Insulin tolerance was not tested in lean females because an additional decrease of preexisting low blood glucose levels causes severe hypoglycemia.

Tumor prevalence and growth were also tested in this model (Fig. 5E). MC38 adenocarcinoma cells (0.5 × 10⁶) were injected into ovx and non-ovx female mice. In this model of pre- vs. postmenopausal obesity, the results also indicate a direct correlation between body adiposity and tumor growth. Tumor size increased 2-fold in obese females and decreased 3-fold in calorie-restricted animals. Ovariectomy resulted in increased adiposity and accelerated tumor growth in all of the groups regardless of their diet.

**Effects of sera from obese animals on tumor cell growth, migration, and colony formation**

The mechanism by which obesity affects tumor growth is still unclear. In human and animal models, sustained positive energy balance results in obesity, which is often accompanied by increased insulin levels and increased fat-produced cytokines and oxidants. In these and perhaps other ways, the milieu for cell metabolism and growth differs profoundly in the obese individual. To explore the effect of insulin, IGF-I and E₂ on H-59-C10 and MC38 cell lines, we tested early signaling events in response to these hormones.
as well as cell proliferation and colony formation. Figure 6A shows that insulin and IGF-I induced AKT phosphorylation in both H-59-C10 and MC38 cell lines at concentrations as low as of 1 and 10 nM, respectively, whereas E2 did not affect AKT or ERK1/2 phosphorylation even at the concentration of 20 nM (estrogen receptor-positive MCF-7 cells served as positive control). A significant increase in ERK1/2 phosphorylation was also evident using the same insulin and

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IGF-I concentrations. To assess the possibility that tumor cell proliferation could be stimulated by insulin, IGF-I or E₂, MC38 and H-59-C10 cells were serum-starved overnight and stimulated with the hormones mentioned above. Cell proliferation was followed over a course of 5 d. As shown in Fig. 6B, H-59-C10 and MC38 cell proliferation was stimulated largely by IGF-I and insulin but was not affected by E₂. These data are consistent with our observation in vivo, in which ovx female mice have an accelerated tumor growth. Fat tissue is a significant source of estrogens that promote growth of number of tumors. The data of the present study, however, demonstrate that accelerated growth of MC38 and H-59-C10 tumor cells in ovx mice under the experimental conditions used, is not attributed to estrogens. This suggests that tumor growth was affected by obesity per se and not by E₂. Interestingly, cell migration induced by sera obtained from obese male mice did not differ significantly from cell migration toward sera obtained from control mice (Fig. 6C). In contrast, anchorage-independent growth of H-59-C10 cells in semisolid agar was significantly enhanced in response to sera from obese mice compared with control sera (Fig. 6D), suggesting that factors present in the sera of obese mice can augment tumorigenicity.

Discussion

This study demonstrates a positive relationship between obesity and tumor growth. Obese animals had approximately a 30% higher rate of palpable tumor development 1 wk after sc injection of cancer cells compared with control mice. Average tumor size was significantly higher in obese than in nonobese animals. These data strongly suggest that tumors cells proliferate more rapidly in animals with higher body fat content.

This study also demonstrates gender differences in response to a high-calorie diet. Both male and female mice became obese and developed glucose intolerance on a high-calorie diet. However, only male mice developed insulin resistance and hyperinsulinemia. Additionally, obese male mice developed more severe glucose intolerance than obese females, and their leptin levels were higher than in obese female mice. These gender differences in insulin sensitivities and leptin levels may originate from gonadal steroids status, i.e. increased serum estrogen in female mice, which has been shown to modulate glucose homeostasis (reviewed in Ref. 30). A study by Clegg et al. (31) demonstrated that estrogen exerts its catabolic actions within the brain by enhancing leptin sensitivity, reducing insulin sensitivity and altering white fat distribution to favor sc fat over visceral fat (31–33). This study suggests that E₂ acts both systemically and centrally, interacts with leptin and insulin in the brain, and modulates the sensitivity to their signal. A previous work by Clegg et al. (34) demonstrated that brains of male rats are more sensitive to the catabolic action of insulin, whereas brains of female rats respond better to the catabolic action of leptin. This, together with estrogen status, may explain the lower levels of leptin in obese females vs. obese males in our study. In the present study, ovariectomy, which is known to mimic the postmenopausal state in mice (23, 24), resulted in increased fat mass, increased leptin levels, and decreased insulin sensitivity. The loss of insulin sensitivity was not due to the ovariectomy itself because insulin responsiveness was normal in ovx females on the control diet. In contrast, glucose tolerance was severely impaired in ovx females regardless of diet. Notably, our results also show that ovariectomy increases adiposity in females regardless of diet, and that increased adiposity correlates with increased tumor growth.

Our histological data revealed that sc tumors were surrounded by adipose tissue in mice fed a high-calorie diet in both genders and that fat depots were also detected in the center of the tumor. Previous studies have shown that adipocytes produce a variety of biologically active cytokines, and these potent small molecules could play a role in stimulating tumor growth. Adipocyte-associated cytokines include plasminogen activator inhibitor-1 (PAI-1), TNF-α, resistin, leptin, adiponectin, and IGF-1, all of which are implicated in cell growth, proliferation, differentiation, cell cycle control, and angiogenesis. Leptin, whose serum concentration correlates with white adipose tissue mass, stimulates angiogenesis that could support tumor growth by promoting development of stromal vasculature (35–37). Adipose tissue is also capable of producing reactive oxygen species, which stimulate expression of PLA-1, IL-6 (38–41), angiotensinogen, and monocyte-chemotactic protein-1 (MCP-1) and reduce genomic stability (42–44). Elevated MCP-1 could increase infiltration of macrophages into adipose tissue, which could promote tumor growth by increasing secretion of growth factors such as vascular endothelial growth factor or epidermal growth factor under hypoxic conditions (45–47). In the current study, we also measured serum MCP-1, IL-6, and TNF-α levels. No significant differences, however, were found between control and obese mice (data not shown).

Increased fat mass leads to increased serum-free fatty acids, which can also modulate tumor growth. Cytotoxic T-lymphocytes play an important role in tumor immunity and are responsible for the clearance of tumor cells. There is evidence that increased free fatty acids prevent cytotoxic T-lymphocyte-mediated killing of tumor cells in vivo (48) and thereby enhance immune suppression, which is essential to attenuate tumor growth. Additional evidence that free fatty acids are essential for tumor growth was demonstrated in breast cancer where inhibition of fatty acid synthase attenuated tumor growth (49). These studies are consistent with the notion that increased levels of free fatty acids are necessary for the rapidly metabolizing cancer cells.

Hormonal status may also affect obesity-associated tumor susceptibility. For example, chronic hyperinsulinemia could activate IGF-I receptor (IGF-IR), thereby stimulating cell growth and inhibiting apoptosis. Alternatively, insulin could mediate its effect through insulin receptor A isoform (IR-A), which has a higher mitogenic potential than insulin receptor B isoform (IR-B) (50). Indeed, Belfiore and colleagues (51–54) have shown that certain cancer cells express more IR-A than IGF-IR. Moreover, insulin can signal through heterodimeric receptors containing IGF-IR and IR. Although we did not demonstrate increased insulin levels in female mice, we demonstrated a state of insulin insensitivity, which might have a local effect. Insulin stimulates downstream activation of AKT and MAPK in cancer cells (Fig. 6 and data not shown), which
are known to promote cell proliferation. The results presented here are consistent with a hormone-dependent mechanism of obesity-associated tumor growth in hyperinsulinemic obese males, but such a mechanism is not consistent with the observed results in non-ovx obese females because serum insulin levels are elevated only slightly in these animals. Therefore, we propose that other obesity-associated factors contribute to obesity-associated tumor prevalence in animals lacking hyperinsulinemia.

Members of the IGF family could also play a role in obesity-related tumor growth. In nonobese individuals, IGF-I and IGF-II circulate at high levels that are neutralized by IGF-binding proteins (55). Some studies suggest that individuals whose IGF-I levels are in the upper quartile of the normal range experience increased risk of prostate, breast, colon, lung, and bladder cancers (56–61). Previously, we demonstrated that reducing circulating IGF-I levels through genetic manipulation inhibited colon and mammary cancer growth and metastasis (62, 63). In obese individuals, hyperinsulinemia may lead to increased local IGF-I activity by lowering levels of IGF-binding protein 1. This might lead to higher bioavailability of IGFs and could increase activation of IGF-IR or IR-A. In the present study, we demonstrate that both insulin and IGF-I are able to induce proliferation of cancer cells. However, in the present models serum insulin and IGF-I cannot solely explain the increased tumor growth observed in obese mice. It is therefore suggested that these two hormones play a role in the increased risk of cancer in obese mice; however, their local action might be of more significance.

In summary, this study clearly demonstrates that obesity is associated with increased transplanted tumor growth in male and female mice. Ovariectomy increases susceptibility to obesity, insulin resistance, and tumor growth in female mice, suggesting that endogenous estrogens together with diet affect body composition, insulin resistance, and tumor development. Additional studies are needed to determine the mechanisms that mediate obesity-associated tumor growth. We will therefore focus future studies on possible roles of insulin/IGF signaling and adipokine-driven processes in obesity-associated tumor growth.

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References


23. Nunez NF, Jelovac D, Macedo L, Berrigan D, Perkins SN, Hursti SD, Barrett JC, Brodie A 2004 Effects of the antiestrogen tamoxifen and the anti-


47. LeBedis C, Chen K, Fallavollita L, Boutros T, Brodt P 2002 Peripheral lymph node tumor cells promote growth and tumorigenicity of breast carcinoma cells through the release of IGF-I and EGF. Int J Cancer 100:2–8


57. Juul A, Main K, Blum WF, Lindholm J, Ranke MB, Skakkebaek NE 1994 The ratio between serum levels of insulin-like growth factor (IGF-I) and the IGF binding proteins (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients. Clin Endocrinol (Oxf) 41:85–93


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