Susceptibility to Induced and Spontaneous Carcinogenesis Is Increased in Fatless A-ZIP/F-1 but not in Obese ob/ob Mice

Vitaly Ablamunits,1 Yehuda Cohen,3 Irina B. Brazeel,4 Harold P. Gaetz,2 Charles Vinson,5 and Simon Klebanov1

1New York Obesity Research Center and 2Anatomic Pathology Corporate, St. Luke’s-Roosevelt Hospital Center, New York, New York; School of Medicine, Stony Brook University Health Sciences Center, Stony Brook, New York; 3Bevelle College, University of California at San Diego, La Jolla, California; and 4Laboratory of Metabolism, National Cancer Institute, NIH, Bethesda, Maryland

Abstract

Obesity is typically associated with increased tumor susceptibility, whereas caloric restriction, a regimen resulting in leanness, inhibits carcinogenesis. The link between adiposity and malignancies suggests that adipose tissue may influence carcinogenesis. An adipose tissue hormone, leptin, could be procarcinogenic because it stimulates proliferation in various tissues and tumor cell lines. Leptin may contribute to the correlation between adiposity and malignancies as its levels are usually increased in obese subjects and reduced by caloric restriction. We hypothesized that leptin deficiency, despite obesity, would inhibit carcinogenesis in leptin-null ob/ob mice and tested this hypothesis in two models: (a) two-stage skin carcinogenesis initiated by 7,12-dimethylbenz(a)anthracene and promoted by phorbol 12-myristate 13-acetate (PMA) and (b) p53 deficiency. Contrary to a typical association between obesity and enhanced carcinogenesis, obese ob/ob mice developed induced skin papillomas and spontaneous p53-deficient malignancies, mostly lymphomas, similarly to their lean littermates. Surprisingly, lipodystrophic (ZIP) mice that had very little both adipose tissue and leptin were highly susceptible to carcinogenesis. Hyperphagia, hyperinsulinemia, and hyperglycemia are unlikely to have contributed significantly to the enhancement of carcinogenesis in ZIP mice because similar hyperphagia, hyperinsulinemic, and hyperglycemic ob/ob mice had normal susceptibility to carcinogenesis. Our data suggest that, in contrast to a well-known correlation between obesity and cancer, the direct effect of adipose tissue may rather be protective. (Cancer Res 2006; 66(17): 8897-902)

Introduction

In humans, excessive body weight and obesity are linked to malignancies in numerous organs of gastrointestinal, reproductive, and hematopoietic systems (1–4). Animal studies also find that diet-induced obesity (5–9) and some forms of genetic obesity (10–16) are associated with increased tumor susceptibility. A significant correlation between adiposity and cancer is suggestive of a direct influence of white adipose tissue on carcinogenesis. However, the mechanisms of how adipose tissue may affect carcinogenesis are poorly understood.

One possibility is that adipose tissue may affect carcinogenesis through adipokine secretion. Leptin may be a plausible candidate to mediate the effects of obesity and caloric restriction on carcinogenesis because it stimulates cellular proliferation in several tumor cell lines from esophagus and prostate (17), breast (17–19), colon (20), and bone marrow (21). At the same time, leptin levels are usually increased in obesity in humans (22, 23) and animals (22, 24, 25).

To explore the effect of leptin deficiency on susceptibility to cancer, we chose the model of induced skin carcinogenesis because skin carcinogenesis is enhanced in obesity (11, 14), making it a likely candidate to be regulated by white adipose tissue. To ensure that the observed phenomena were not confined only to the skin, we also used p53-deficient mice, which die of spontaneous malignancies by 10 months (26).

To test whether leptin deficiency would reduce susceptibility to cancer, we used Lep+/ob/obTrp53tm1Tyj/tm1Tyj (ob/ob) mice lacking leptin because of a mutation in the leptin gene (27). ob/ob mice are very obese; hence, potentially beneficial effects of the lack of leptin on carcinogenesis might be disguised in these mice by the negative effects of obesity. We therefore also tested the effect of leptin deficiency on carcinogenesis in lipodystrophic, A-ZIP/F-1 (ZIP) mice that almost completely lack leptin and white adipose tissue (28, 29).

Materials and Methods

Experimental animals and experimental groups. All mice were housed in a specific pathogen-free facility, fed ad libitum with LabDiet 5001 with 4% fat (PMI Nutrition International, Brentwood, MO), and maintained on a 14:10 light-dark cycle, with lights on at 7:00 a.m. and off at 9:00 p.m. Animal studies followed the guidelines of the American Association for Accreditation of Laboratory Animal Care and were approved by the Institutional Animal Care and Use Committee of St. Luke’s-Roosevelt Hospital Center.

To study how leptin deficiency affects susceptibility to skin carcinogenesis, we used obese B6.V-Lep+/ob/obTrp53tm1Tyj/tm1Tyj (control) females purchased from The Jackson Laboratory (Bar Harbor, ME). To study how lipodystrophy affects susceptibility to skin carcinogenesis, we bred C57BL/6j (B6) females with FVB-Tg(A-ZIP/F-1)Vsn/J males, hemizygous by A-ZIP/F-1 transgene (28), and used lipodystrophic (B6 × FVB)F1, Tg(A-ZIP/F-1; ZIP) mice (control) and normal (B6 × FVB)F1 (control) males. All mice were 8 to 11 weeks of age at the beginning of the study.

To study how leptin deficiency affects susceptibility to skin carcinogenesis, we used obese B6.V-Lep+/ob/obTrp53tm1Tyj/tm1Tyj and lean Lep+//-Tg53tm1Tyj/Tyj males on the B6 genetic background. These mice were obtained through several stages of breeding starting with B6.V-Lep+/ob/obTrp53tm1Tyj/Tyj and B6.129S2-Trp53tm1Tyj/-. These mice were obtained from The Jackson Laboratory. To study how lipodystrophy affects susceptibility to malignancies caused by p53 deficiency, we used lipodystrophic Tg(A-ZIP/F-1)Trp53tm1Tyj/Tyj and their normal nontransgenic Trp53tm1Tyj/Tyj littermate males. These mice were obtained through several steps of breeding...
starting with B6.129S2-Trp53tm1Tyj/J females and FVB-Tg(A-ZIP/F-1)1Vsn/J males. The genetic background of all experimental animals was the second backcross (N3) to the B6 strain. Additionally, some non-p53-null mice were studied (Trp53^tm1Tyj^-/-) for the leptin deficiency study and Trp53^+/+ for the lipodystrophy study) to assess the mortality from nonmalignancy causes.

Mice were genotyped according to the protocols provided at The Jackson Laboratory Web site.6 Study of induced skin carcinogenesis. Because experimental animals differed in body size (e.g., ob/ob mice were larger than their lean controls), we tried to ensure that the equal-area skin patches were subjected to carcinogenesis in all groups. All mice were shaved in the dorsal area near their tail and tattooed to mark a circular skin area of 1.5 cm in diameter. Two weeks later, 50 μg 7,12-dimethylbenz(a)anthracene (DMBA) dissolved in 200 μL acetone was applied to the marked skin patch. Another 2 weeks later, mice started to receive applications of phorbol 12-myristate 13-acetate (PMA), 4 μg in 50 μL acetone twice weekly for 25 weeks. Papillomas were observed weekly and those larger than 1 mm in diameter and present for 2 consecutive weeks were scored positive. At the end of the study, the mice were sacrificed and the papillomas were collected for histology.

Skin response to PMA was measured in naive ZIP and control mice. For that, four applications of 4 μg PMA in 50 μL acetone were applied over the period of 2 weeks and epidermal thickness was measured histologically on parallel sections stained with H&E.

Study of spontaneous carcinogenesis in p53 deficiency. All experimental p53-null mice were weaned at 3 weeks of age and observed daily after weaning. Mice that had large tumors (>1 cm in diameter) or were clearly moribund were sacrificed. The rest were allowed to die naturally. All mice were necropsied and visible tumors, spleen, and liver were collected for histology.

Body weight and food consumption measurements. Body weights were measured at 6 months in the skin carcinogenesis study and at 15 weeks in the p53 deficiency study.

Food intake in the skin carcinogenesis study was measured in the same mice that were subjected to the carcinogenesis protocol. In the p53 deficiency study, food intake of p53-null mice might have been affected by occult malignancies. We therefore measured food intake in p53^+/+ (ob/ob groups) or p53^-/- (ZIP groups) littermates of the p53-null mice. Food intake for each group was measured daily in two to five separate cages, with three to five mice per cage, by weighing the food remaining in the food hopper. The measurements were done at 6 months of age.

Body composition measurements. Body composition was measured at 4 months of age, using dual energy X-ray absorptiometry (DEXA; Lunar PIXimus2, Lunar Corp., Madison, WI) under isoflurane anesthesia. Lean mass, fat mass, and percent fat were calculated using the software provided by the manufacturer.

Blood hormone and metabolite measurements. Blood samples were collected at 6 months of age in the skin carcinogenesis study and at 15 weeks of age in the p53 deficiency study. Blood glucose levels were measured by a FreeStyle blood glucose monitoring system (TheraSense, Inc., Alameda, CA). Plasma corticosterone levels were determined by a double-antibody corticosterone RIA from diagnostic laboratories (St. Charles, MO). Plasma total and adiponectin and insulin levels were assessed by mouse adiponectin and insulin RIA kits from Matersenase Diagnostics (Salem, NH). Plasma leptin was determined by ELISA using mouse Leptin DuoSet kit from R&D Systems, Inc. (Minneapolis, MN).

Statistics. Food intake, body weight, hormone, and metabolite levels and skin papilloma numbers were expressed as mean ± SE and analyzed by the two-tailed t test. Papilloma incidence was analyzed by the χ^2 test. Survival analysis was done using the log-rank Kaplan-Meier estimation. Significance level was set at P < 0.05.

Results

Susceptibility to skin carcinogenesis is not increased in leptin-deficient ob/ob mice. ob/ob mice are extremely obese. Body composition measured by DEXA revealed a significant difference between ob/ob and lean B6 controls: lean mass, fat mass, and percent fat were 27.1 ± 0.5 g, 44.8 ± 1.2 g, and 62.3 ± 0.6% in ob/ob mice compared with 20.3 ± 0.6 g, 5.2 ± 0.4 g, and 20.2 ± 3.9% in lean controls, respectively.

To test whether leptin regulates susceptibility to skin carcinogenesis, leptin-deficient ob/ob mice were treated with DMBA and, beginning 2 weeks later, they were treated with PMA twice weekly for 25 weeks. The percentage of mice bearing papillomas and the average papilloma number are shown on Fig. 1A and B, respectively. Neither the incidence nor average tumor number differed between lean control and ob/ob mice. The incidence and the timing of onset in control B6 mice were comparable with those reported previously for this strain (30, 31).

Susceptibility to skin carcinogenesis is significantly increased in lipodystrophic ZIP mice. ob/ob mice lack leptin and are therefore obese. As obesity is linked to increased cancer susceptibility, including skin cancer (6, 11, 14), it could have disguised the beneficial effect of the lack of leptin in ob/ob mice. We therefore tested whether lipodystrophic ZIP mice (28, 29), for which we confirmed both leptin levels below 5% of those in controls (Table 1) and the lack of visible adipose tissue, would be less susceptible to skin carcinogenesis than their normal littermates.

We used the same carcinogenesis protocol as with ob/ob mice, a single application of DMBA followed by 25 weeks of PMA applications. The percentage of mice bearing papillomas and the average papilloma number are shown on Fig. 1C and D, respectively. Surprisingly, lipodystrophic mice developed tumors significantly earlier than control mice. They also developed significantly more tumors per animal. Representative papillomas in control and lipodystrophic mice after 25 weeks of PMA treatment are shown on Fig. 2A and B, respectively.
ZIP papillomas are better vascularized and epidermis of ZIP mice is more responsive to PMA. We tested whether ZIP mouse papillomas were histologically different from control mouse papillomas and found that they had a significantly higher capillary density (Fig. 2C and D). This difference could explain a faster papilloma growth in ZIP mice.

To explain why lipodystrophic ZIP mice have high susceptibility to induced skin carcinogenesis, we hypothesized that their skin was more responsive to PMA treatment. Indeed, the growth response of the epidermis to four PMA applications over a 2-week period was much more pronounced in lipodystrophic ZIP than in normal control mice (Fig. 3A-D). This difference in the responsiveness to PMA could explain a significantly accelerated papilloma appearance in ZIP mice.

Hormones that may affect susceptibility to carcinogenesis. As increased capillary density might have contributed to the accelerated papilloma growth in lipodystrophic mice, we measured the levels of adiponectin, a hormone, produced by white adipose tissue and suggested to be antiangiogenic (32). Adiponectin levels in lipodystrophic ZIP mice were below the detection limit (i.e., below 8% of those in control mice; Table 1). Adiponectin levels in both control ZIP and ob/ob mice were below the detection limit of our ELISA (i.e., <0.15 ng/mL, that is <5% of the respective control levels). Thus, residual leptin in ZIP is unlikely to explain accelerated papilloma growth in ZIP mice.

Table 1. Plasma hormone levels

<table>
<thead>
<tr>
<th></th>
<th>Obesity</th>
<th>Lipodystrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>15.1 ± 1.7 (7)*</td>
<td>&lt;0.8 (3)*</td>
</tr>
<tr>
<td>IGF-I (ng/mL)</td>
<td>209 ± 15 (5)</td>
<td>10.3 ± 0.5 (5)</td>
</tr>
<tr>
<td>Corticosterone (ng/mL)</td>
<td>421 ± 49 (5)*</td>
<td>243 ± 20 (5)</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>&lt;0.15 (5)*</td>
<td>243 ± 39 (12)*</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>30.5 ± 6.6 (4)*</td>
<td>&lt;0.15 (4)*</td>
</tr>
</tbody>
</table>

NOTE: Mice were bled at 6 months of age and plasma hormones were measured as described in Materials and Methods. Data are mean ± SE (number of animals tested).

*p < 0.05, compared with respective controls.
a substantial difference in skin tumor incidence between ZIP and ob/ob mice.

Lipodystrophy shortens life span of carcinogenesis prone p53 knockout mice. A significant acceleration of carcinogenesis in the skin suggests that lipodystrophy may have a generalized promoting effect on malignancy. To test this hypothesis, we used mice deficient in the tumor suppressor p53 gene that spontaneously develop malignancies and die by 10 months of age (26, 43). We tested four groups of p53-null mice, ob/ob and their littermate controls and lipodystrophic ZIP and their littermate controls. Approximately 80% of mice in all groups died of lymphomas and thymomas. There was no significant difference between the groups in the variety and relative frequency of malignancies.

Longevity was not affected by obesity in leptin-deficient ob/ob mice (Fig. 4A). Life span of lipodystrophic ZIP mice was, however, significantly shorter (P < 0.05) than that of control mice, 136 ± 6 and 160 ± 10 days, respectively (Fig. 4B). Survival of lipodystrophic and control mice was also different when analyzed using the log-rank Kaplan-Meier estimation (P < 0.05).

Hyperphagia and hyperglycemia are present not only in ZIP but also in ob/ob mice and therefore are likely not the reason for an increased susceptibility to carcinogenesis in ZIP mice. Because caloric restriction inhibits induced and spontaneous carcinogenesis (44–47), we explored the possibility that hyperphagia and hyperglycemia characteristic of lipodystrophic ZIP mice (28) could be responsible for the enhanced susceptibility to carcinogenesis. In the two studies discussed above, on induced skin carcinogenesis and on spontaneous carcinogenesis in p53-deficient mice, ZIP mice were hyperphagic by 2.1- and 1.5-fold, respectively, and hyperglycemic by 2.7- and 1.6-fold, respectively, compared with their normal littermates (Fig. 5). However, ob/ob mice were similarly hyperphagic, by 1.6- and 1.5-fold, and hyperglycemic, by 2.1- and 1.6-fold, compared with their normal littermates (Fig. 5). As reported above, ob/ob, in contrast to ZIP mice, were not, however, more carcinogenesis susceptible than their normal littermates. Thus, probably, neither hyperphagia, nor hyperglycemia, nor hyperinsulinemia (Table 1) per se contributed significantly to enhanced carcinogenesis in lipodystrophic ZIP mice.

Discussion

Skin carcinogenesis is enhanced in genetically obese Ay mice (11, 14) and in normal mice made obese by feeding a high fat diet (6). Here, we tested the hypothesis that leptin, which is increased in obesity (22–25) and which has a direct stimulatory effect on skin cells in vivo (39, 40) and in vitro (40–42), was responsible for the effects of obesity on the susceptibility to skin carcinogenesis. We found that leptin deficiency does not make ob/ob mice less susceptible than leptin-replete lean control mice. To test the possibility that a suppressive effect of the lack of leptin on skin carcinogenesis was disguised in ob/ob mice by their extreme obesity, we also tested whether skin carcinogenesis was suppressed in hypo leptinemic ZIP mice with very little white adipose tissue.
(28, 29). Contrary to our expectation, the lack of adipose tissue enhanced skin carcinogenesis.

When tested in another carcinogenesis model, p53 knockout mice, leptin deficiency again had no effect, whereas lipodystrophy significantly shortened life span of these malignancy-prone mice. Thus, lipodystrophy, in contrast to hypoleptinemic obesity, had a strong promoting effect on both induced skin and p53 deficiency carcinogenesis.

The enhancement of carcinogenesis in ZIP mice is unlikely due to some nonspecific effects of insulin resistance, diabetes, and hyperphagia as these phenomena are also present in ob/ob mice, which are not more susceptible to skin carcinogenesis than lean control mice.

Two hormones, IGF-I and corticosterone, known, respectively, to enhance (33, 34, 48) and suppress (35, 36, 49) carcinogenesis also cannot be responsible for the major difference between ob/ob and ZIP mice: IGF-I levels were not affected by either leptin deficiency or lipodystrophy and corticosterone levels were similarly elevated in both ob/ob and ZIP mice.

Most studies that find a positive relationship between adiposity and carcinogenesis have not been designed to test whether the relationship between obesity and cancer is causal (4, 7, 8, 10–14, 16). In such studies, the adiposity was not an independent variable itself and the connection between adiposity and carcinogenesis, thus, might have arisen because both were regulated by the same underlying factor(s). In contrast, in the ZIP mouse, the primary effect causing lipodystrophy, the expression of a dominant-negative A-ZIP/F-1 transcription factor, is confined to preadipocytes/adipocytes themselves (28). Therefore, our finding that lipodystrophy enhances skin carcinogenesis unequivocally shows that white adipose tissue may indeed have a significant negative effect on carcinogenesis. To reconcile this apparently counterintuitive conclusion with numerous reports about positive correlation between adiposity and carcinogenesis, we propose that adipose tissue produces a carcinogenesis-suppressing factor, whose levels are decreased in obesity and elevated by caloric restriction (55, 56), a condition known to inhibit carcinogenesis (44–47). Although adiponectin was only somewhat suppressed in ob/ob mice, it was undetectable in ZIP mice (Table 1). Adiponectin is anticarcinogenic (32) and its absence in ZIP mice might have contributed to enhanced carcinogenesis. Its antiangiogenic activity (32) might have also been responsible for a significantly increased vascularization of papillomas in ZIP mice.

Interleukin-1 (IL-1) receptor antagonist, an anti-inflammatory protein secreted by adipose tissue (57), may be another adipokine with an anticarcinogenic effect (58, 59). However, the role of IL-1/IL-1 receptor/IL-1 receptor antagonist system in carcinogenesis has not been fully settled (60). Adipose tissue secretes a large number of adipokines (61) and their role in carcinogenesis will have to be addressed in future studies.

Our study shows that lipodystrophy enhances skin carcinogenesis and accelerates the development of lymphomas, thus establishing a potentially important role for adipose tissue as a modulator of carcinogenesis. One of the hormones mediating this effect of white adipose tissue may be adiponectin. Although the lack of an effect in ob/ob mice suggests that leptin reduction is not involved in mediating the inhibitory effect of caloric restriction on skin and hematopoietic carcinogenesis, the role of leptin in mediating the effects of caloric restriction and obesity on carcinogenesis in other tissues cannot be ruled out (62–64). Finally, abnormal lipid metabolism in adipose tissue may have a direct effect on lipid metabolism in other tissues, leading to ectopic lipid accumulation and altered cancer susceptibility. Further studies of the endocrinology and metabolism of adipose tissue are required to clarify the nature of the relationship between obesity and carcinogenesis.

Acknowledgments

Received 1/16/2006; revised 5/16/2006; accepted 6/12/2006.

Grant support: NIH AG 19894 (S. Klebanov).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
References

Susceptibility to Induced and Spontaneous Carcinogenesis Is Increased in Fatless A-ZIP/F-1 but not in Obese $ob/ob$ Mice


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/66/17/8897

Cited articles  This article cites 63 articles, 23 of which you can access for free at: http://cancerres.aacrjournals.org/content/66/17/8897.full.html#ref-list-1

Citing articles  This article has been cited by 4 HighWire-hosted articles. Access the articles at: /content/66/17/8897.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.