Accelerated Tumor Formation in a Fatless Mouse with Type 2 Diabetes and Inflammation

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Abstract

Epidemiologic studies show a positive association between obesity and cancer risk. In addition to increased body adiposity and secretion of fat-derived hormones, obesity is also linked to insulin resistance, type 2 diabetes, and chronic inflammation. We used the fatless A-ZIP/F-1 transgenic mouse to dissociate the relative role of each of these underlying factors in the development of cancer. These mice are unique in that they do not have white fat but do develop type 2 diabetes. In two cancer models, the classic two-stage skin carcinogenesis protocol and the C3(1)/T-Ag transgenic mouse mammary tumor model, A-ZIP/F-1 mice displayed higher tumor incidence, tumor multiplicity, and decreased tumor latency than wild-type mice. We examined circulating levels of adipokines, growth factors, and cytokines. As expected, adipokines (i.e., leptin, adiponectin, and resistin) were undetectable or found at very low levels in the blood of fatless mice. However, insulin, insulin-like growth factor-I, growth hormone, vascular endothelial growth factor, and proinflammatory Th2 cytokines, such as interleukin (IL)-1β, IL-4, and IL-6, were elevated in A-ZIP/F-1 mice. Additionally, we examined multiple phosphorylated proteins (i.e., protein kinase B/Akt and ErbB2/HER-2 kinase) associated with cancer development. Results show that many of these phosphorylated proteins were activated specifically in the A-ZIP/F-1 skin but not in the wild-type skin. These findings suggest that adipokines are not required for the promotion of tumor development and thus contradict the epidemiologic data linking obesity to carcinogenesis. We postulate that insulin resistance and inflammation are responsible for the positive correlation with cancer observed in A-ZIP/F-1 mice. (Cancer Res 2006; 66(10): 5469-76)

Introduction

Obesity has increased dramatically over the past 20 years in the United States (1) and in other developed countries and is associated with increased incidence (2, 3) and mortality rates (4) of various cancers. In the United States, excess body weight has been implicated in ~20% of all cancer deaths in women and 14% in men or ~90,000 cancer deaths yearly (4). Unfortunately, the mechanism by which obesity increases cancer risk is not yet known. Insights into the mechanism(s) through which obesity increases the risk of cancer are urgently needed to develop new strategies for the prevention and treatment of certain cancers.

Current epidemiologic and experimental studies of the obesity-cancer association focus on the role of increased adipose tissue, particularly the increase in circulating adipocyte-derived factors (adipokines). Multiple factors from adipose tissue, such as leptin, adiponectin, cytokines, and other secreted products, influence processes involved in carcinogenesis (5, 6). In addition, reduction of adiposity by calorie restriction (7, 8) or physical activity suppresses tumor development (9) and lowers circulating levels of many adipokines, further implicating them in tumor development.

Suggested mechanisms underlying the obesity and cancer association include the excess production of biologically active adipokines (6, 10), the induction of insulin resistance (11), or the promotion of inflammation (10, 12). Unfortunately, the relative role each of these factors plays in cancer development in obese individuals is not yet known. To uncouple the effects of adipose tissue from diabetes and inflammation on cancer progression, we used the diabetic A-ZIP/F-1 transgenic mouse, which lacks white adipose tissue but is profoundly insulin resistant and hyperlipidemic (13). Additionally, these mice display a state of inflammation as indicated by the high systemic levels of inflammatory cytokines. Therefore, the A-ZIP/F-1 fatless mouse is a unique laboratory tool that can be used to uncouple the effects of adipose tissue from those of type 2 diabetes/insulin resistance and inflammation on cancer development. In two different cancer models, we found that A-ZIP/F-1 mice are more prone to develop tumors as indicated by their higher tumor incidence, tumor multiplicity, and decreased tumor latency than wild-type mice. The increased cancer susceptibility of the A-ZIP/F-1 mice is accompanied by a profound type 2 diabetes/insulin resistance and inflammatory state. Therefore, these findings suggest that adipose tissue or adipokines may not be required for the promotion of tumor development by obesity.

Materials and Methods

Animals. The generation of A-ZIP/F-1 transgenic mice has been described previously (13). The following is a brief description of these mice. Under the control of the adipose-specific aP2 enhancer/promoter, these mice express A-ZIP/F-1, a dominant-negative protein that prevents the DNA-binding of B-ZIP transcription factors of both C/EBP and Jun families. Prevention of the DNA-binding of the B-ZIP transcription factors inhibits adipogenesis, which leads to a fatless phenotype. In addition to lacking white fat, A-ZIP/F-1 mice have dramatically reduced amounts of

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Table 1. Growth of A-ZIP/F-1 and wild-type mice

Age (wk)	Group (n = 6)	Body length (cm)	Body weight (g)	Liver (g)	Triglycerides (mg/dL)	Free fatty acids (mmol/L)
4	Wild-type	6.90 ± 0.07	17.0 ± 0.33	0.93 ± 0.04	172 ± 20	0.30 ± 0.03
	A-ZIP/F-1	6.15 ± 0.09*	14.2 ± 0.60*	1.17 ± 0.07*	403 ± 32*	0.60 ± 0.05*
8	Wild-type	7.32 ± 0.14	20.3 ± 0.50	1.01 ± 0.11	280 ± 20	0.37 ± 0.03
	A-ZIP/F-1	7.60 ± 0.12	22.40 ± 0.40*	2.13 ± 0.12*	490 ± 22*	0.60 ± 0.03*
12	Wild-type	7.30 ± 0.05	23.3 ± 0.69	0.90 ± 0.15	187 ± 17	0.23 ± 0.020
	A-ZIP/F-1	7.50 ± 0.08*	24.52 ± 0.69	2.17 ± 0.10*	585 ± 37*	0.53 ± 0.02*
16	Wild-type	7.40 ± 0.06	22.6 ± 0.40	1.15 ± 0.03	ND	ND
	A-ZIP/F-1	8.20 ± 0.09*	27.8 ± 0.80*	2.56 ± 0.18*	ND	ND

NOTE: The difference in triglycerides and free fatty acid levels between A-ZIP/F-1 and wild-type mice was similar at 4, 8, and 12 weeks of age.
Abbreviation: ND, not done.
*Significantly different from wild-type mice ($P \leq 0.05$). See Statistical analysis.

brown adipose tissue, which is inactive (13, 14). A-ZIP/F-1 mice initially are growth-delayed but surpass their littermates in weight by week 12 (13). These mice eat, drink, and urinate copiously and have decreased fecundity. The mice also have fatty liver, are diabetic, and have elevated systemic glucose, insulin, free fatty acids, and triglycerides. Furthermore, the A-ZIP/F-1 mice have a rudimentary mammary anlagen form, which are unable to grow and branch normally, resulting in a few, short, severely distended ducts (15). However, when mammary glands from A-ZIP/F-1 mice are placed into a normal stromal environment of syngeneic mice, the epithelial cells from A-ZIP/F-1 mice possess the potential for normal growth and differentiation (15).

We also used the C3(1)/T-Ag transgenic mouse as a model for breast cancer (16). In this breast cancer mouse model, the expression of the SV40 large tumor antigen transforming sequences results in spontaneous mammary tumors (11). The A-ZIP/F-1 and C3(1)/T-Ag transgenic mice are both of the FVB/N genetic background. C3(1)/T-Ag transgenic mice were obtained from the National Cancer Institute (NCI; Frederick, MD).

Blood samples were drawn from the retro-orbital venous plexus of lightly anesthetized mice (Metafane, Mallinckrodt Veterinary, Inc., Mundelein, IL). Serum was separated by centrifugation at 14,000 rpm for 5 minutes (Marathon 13k/12, Fisher Scientific, Pittsburgh, PA) and stored at −70°C until analyses. All procedures involving animals were approved and monitored by the NCI Animal Care and Use Committee. Animal care was provided in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (NIH Publication 86-23, 1985).

Skin carcinogenesis. For this purpose, 8-week-old female A-ZIP/F-1 and wild-type FVB/N mice were shaved on the dorsal skin and treated with a single topical application of 7,12-dimethylbenz(*a*)anthracene (DMBA; 25 μg in 200 μL acetone). After 7 days, the mice received biweekly applications of 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 5 μg in 200 μL in acetone) for 20 weeks to induce papillomas (17). Mice were monitored weekly for tumor onset, and their growth was measured using calipers. Tumors and skin were collected and fixed in 10% buffered formalin and embedded in paraffin; sections were prepared and stained for histologic analysis.

Mouse model of breast cancer. We examined whether A-ZIP/F-1 mice had an altered susceptibility of developing mammary tumors by crossing them to a genetic model of breast cancer. Female homozygous C3(1)/T-Ag transgenic mice were crossed to male A-ZIP/F-1 mice that are hemizygous for the transgene. All female progeny from this cross are hemizygous for C3(1)/T-Ag, and half of the females contain the A-ZIP/F-1 transgene. We compared tumor incidence in these two genotypes. The tumor volume was determined by measuring the length, width, and depth of the tumor using calipers. Tumors were harvested for histologic analyses when they reached at least 1.5 cm^2. Whole mounts were done as described previously (16).

Biochemical assays. Serum glucose, triglycerides, nonesterified fatty acids, and insulin were assayed as described previously (18).

Adipokines. Given that fatless A-ZIP/F-1 mice have no white fat, adipokines, such as leptin, should be extremely low or undetectable. To verify that this was the case, adipokines were measured in the serum of wild-type and A-ZIP/F-1 transgenic mice. Serum leptin, resistin, and adiponectin measurements were done using 10 μL serum in 14-week-old female mice by the Luminex-based bead array method using a LINCOplex simultaneous multianalyte detection system (Linco Research, Inc., St. Charles, MO) according to the manufacturer's instructions.

Insulin-like growth factor-I and growth hormone. Animal studies have shown that insulin-like growth factor-I (IGF-I) and growth hormone influence the susceptibility of animals to mammary cancers (19, 20). To evaluate the IGF-I and growth hormone levels in A-ZIP/F-1 mice, we examined their concentrations in the serum. Growth hormone (in 24-week-old mice) and IGF-I (in 14-week-old mice) were measured with commercially available RIA kits from Linco Research and Diagnostic Systems Laboratories, Inc. (Webster, TX).

Serum markers of inflammation. To determine if the diabetic A-ZIP/F-1 female mice have an inherent inflammatory state, we measured the following serum cytokines: interleukin (IL)-1β, IL-2, IL-4, IL-5, tumor necrosis factor-α (TNF-α), granulocyte macrophage colony-stimulating factor (GM-CSF), IL-6, IL-10, IL-12p40, IL-12p70, IL-13, IFN-γ, MCP-1, and vascular endothelial growth factor (VEGF). Cytokine measurements were done by Allied Biotech, Inc. (Ijamsville, MD) using a mouse cytokine microarray kit.

"Kinetworks" phosphoprotein analysis. We measured a large number of phosphoproteins in the skin of wild-type and A-ZIP/F-1 mutant mice to determine their association with serum hormone, adipokines, and cytokine levels. For this purpose, the Kinexus phospho-antibody screening system was used. Protein samples from the skin of wild-type and A-ZIP/F-1 mutant mice at the end of the skin carcinogenesis protocol (week 20) were prepared as recommended by Kinexus Bioinformatics Corp. (21).[7] The panel of target phosphoproteins detected by the Kinetworks KPSS-1.3, KPSS-11, and KPSS-12 screens are listed in the Kinexus Web site.

Statistical analysis. All statistical analysis was done using JMP 5.0 (SAS Institute, Inc., Cary, NC). Age-specific changes in body length, body weight, liver weight, serum free fatty acids, and serum triglycerides were analyzed by ANOVA with *a posteriori* comparison of the means using Tukey's Honestly Significant Difference procedure. Note that different animals were examined at each time point. Distributions of triglycerides and free fatty acids deviated somewhat from normality, but analysis of natural log-transformed data did not alter the results. Significant effects are reported at $P \leq 0.05$. Skin papilloma number, skin tumor volume, and mammary tumor volumes at 16, 14, and 130 days, respectively, were compared

[7] http://www.kinexus.ca.

between wild-type and A-ZIP/F-1 mice using *t* tests. Mammary tumor incidence at 11, 120, and 130 days were compared between the two groups using nominal logistic regression with age as a covariate. All remaining comparisons were done using *t* tests on untransformed data.

Results

Phenotype of A-ZIP/F-1 mice. The body size phenotype and diabetic sequelae of A-ZIP/F-1 mice have been extensively described (13, 18). Herein, we measured previously calculated variables associated with the growth and diabetic phenotype of these mice (Table 1). As reported previously, A-ZIP/F-1 mice were initially growth-delayed but surpassed their littermates in body weight after age 12 weeks (13). At age 4 weeks, A-ZIP/F-1 mice weighed less, and their body length was shorter than their littermates (Table 1). However, at age 16 weeks, A-ZIP/F-1 mice surpassed their littermates in both body weight and body length (Table 1). A-ZIP/F-1 mice were hyperlipidemic, as indicated by the high systemic levels of triglycerides and free fatty acids (Table 1). Moreover, the diabetes in the A-ZIP/F-1 mice is associated with an enlarged liver engorged with lipid (Table 1). In summary, results in Table 1 show similar findings as reported previously in the A-ZIP/F-1 mice; these mice are hyperlipidemic, hyperinsulinemic, and hyperglycemic.

Increased skin carcinogenesis in the "fatless" mutant A-ZIP/F-1 mouse. The first carcinogenic model we examined was the classic two-stage skin carcinogenesis bioassay, which involves a single topical application of DMBA to the dorsal flank of 8-week-old mice, followed by biweekly applications of TPA for 20 weeks to induce papillomas on the skin (17). This protocol did not change the elevated glucose or insulin levels in A-ZIP/F-1 mice (data not shown). A-ZIP/F-1 mice developed larger and more numerous papillomas sooner than wild-type mice (Fig. 1*A* and *B*). The morphology of papillomas was similar between A-ZIP/F-1 transgenic and wild-type mice. There were no significant differences in cell proliferation, vascularity, or macrophage infiltrates, which were examined by staining for Ki-67, CD31, and F480, respectively (data not shown). Figure 2 shows a photograph of the dorsal view of the skin papillomas developed in A-ZIP/F-1 and wild-type female mice.

Mammary cancer in the absence of adipose tissue. To determine the effects of the fatless phenotype on mammary cancer development, homozygous C3(1)/T-Ag transgenic female mice were crossbred with hemizygous A-ZIP/F-1 transgenic male mice to produce two genotypes, C3(1)/T-Ag and C3(1)/T-Ag:A-ZIP/F-1 mice. Expression of the SV40 large tumor antigen in the mammary epithelium of C3(1)/T-Ag mice promotes development of mammary tumors (16). Results show that mice in the A-ZIP/F-1

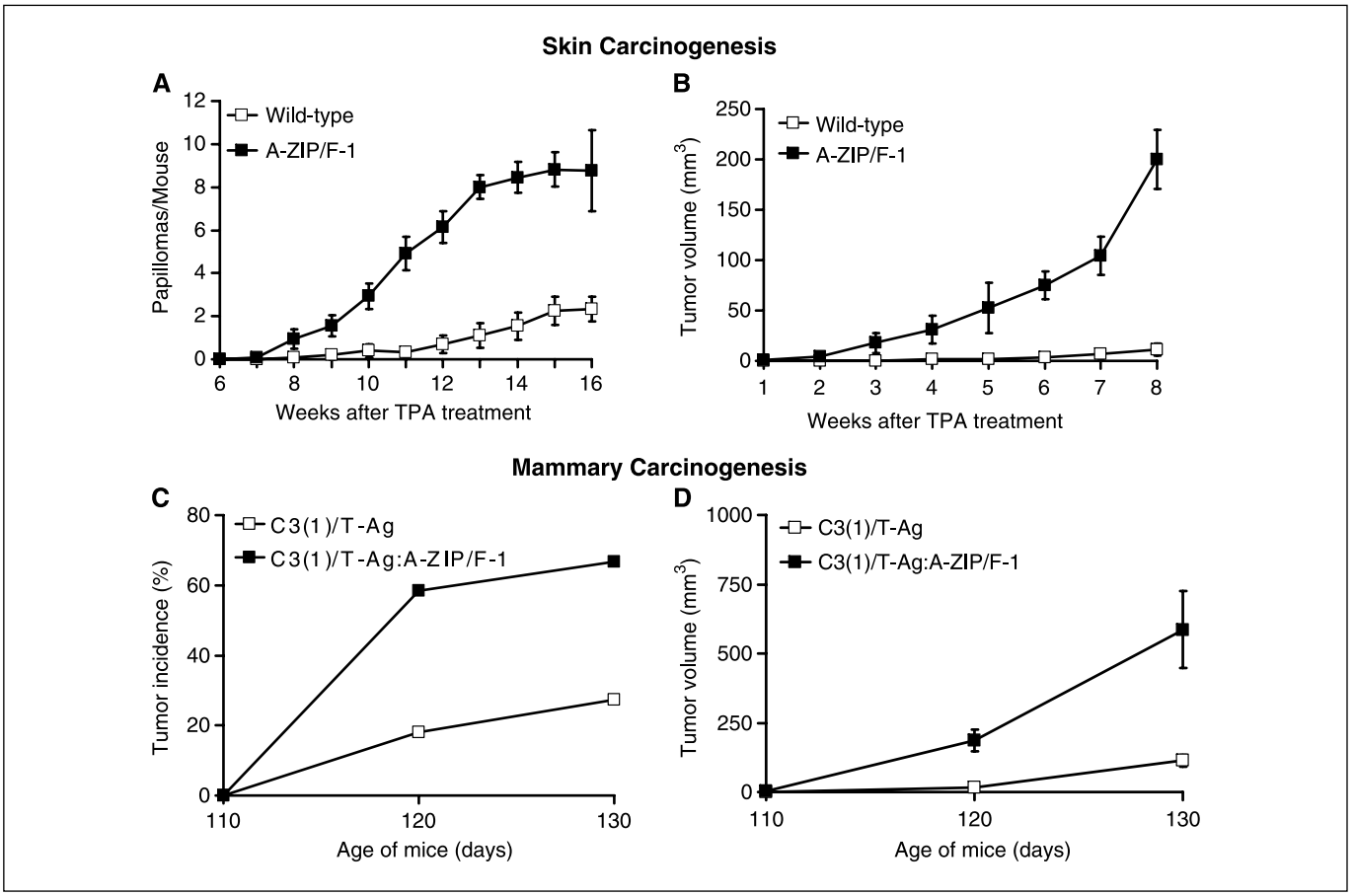

Figure 1. Skin carcinogenesis in A-ZIP/F-1 fatless and wild-type mice (*A* and *B*). ■, A-ZIP/F-1 fatless ($n = 14$); □, wild-type mice ($n = 10$). *A,* number of papillomas per mouse. *B,* tumor volume. *Points,* average; *bars,* SD. Genetically-induced mammary tumors (*C* and *D*). *C,* incidence of tumors monitored over time in heterozygous C3(1)/T-Ag transgenic female mice in the presence (■; $n = 12$) or absence (□; $n = 14$) of the A-ZIP/F-1 transgene. Results are percent of mice with tumors over time. *D,* tumor volume over time. *Points,* average; *bars,* SD. *, $P \leq 0.05$, C3(1)/T-Ag:A-ZIP/F-1 mice have significantly higher tumor incidence and higher tumor growth rate than the wild-type mice.

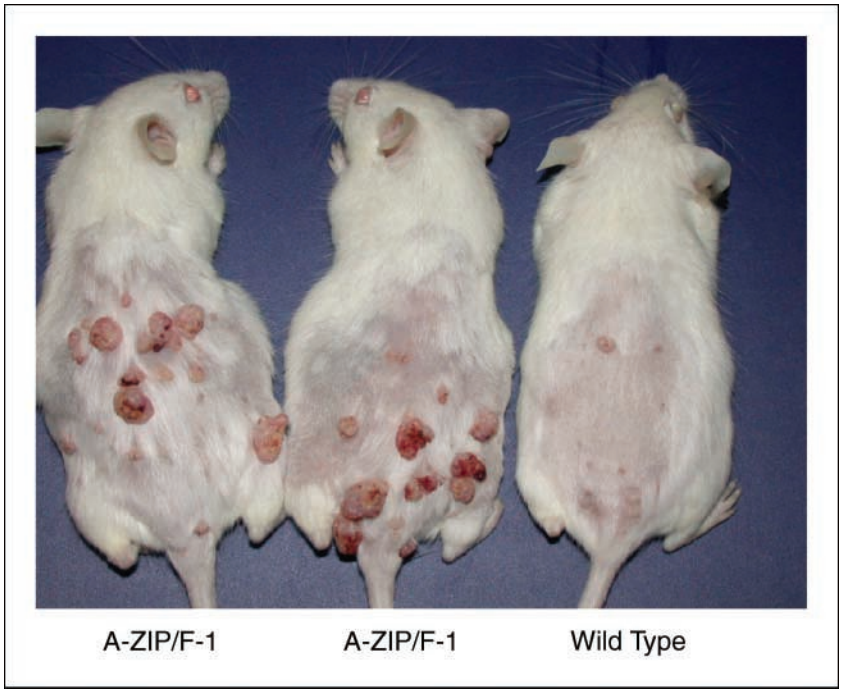

Figure 2. Photograph of two A-ZIP/F-1 and one wild-type female littermate mice 14 weeks after treatment with DMBA. Note the presence of numerous papillomas in the transgenic A-ZIP/F-1 mice but not in the wild-type mouse.

background developed more numerous and larger tumors more quickly (Fig. 1*C* and *D*). For example, by 120 days, 64% of the C3(1)/T-Ag:A-ZIP/F-1 mice, and only 27% of the C3(1)/T-Ag mice, had developed tumors ($P < 0.05$; Fig. 1*C* and *D*). Expression of the SV40 large tumor antigen in the mammary epithelium of A-ZIP/F-1 mice did not change the diabetic phenotype of the A-ZIP/F-1 mice (data not shown).

Mammary tumors from either C3(1)/T-Ag transgenic mice or C3(1)/T-Ag:A-ZIP/F-1 mice were harvested when mice were burdened by a 1.5 cm^2 tumor. The histology of tumors from C3(1)/T-Ag mouse mammary model has been described previously (16). The majority of tumors were anaplastic carcinomas with a mixture of architectures: glandular, solid, and papillary. However, C3(1)/T-Ag:A-ZIP/F-1 transgenic mice had a wider spectrum of carcinoma types compared with C3(1)/T-Ag mice (Table 2). Malignant adenomyoepitheliomas occurred in 3 of 13 C3(1)/T-Ag:A-ZIP/F-1 mice and 2 of 13 C3(1)/T-Ag mice as identified by smooth muscle actin antibody immunostaining (data not shown). Figure 3 shows histology of mammary tumors in both genetic backgrounds. In addition, tumors in C3(1)/T-Ag:A-ZIP/F-1 mice often arose in unusually dilated ducts. Immunoreactivity for Ki-67, CD31, and collagen (Masson's trichrome) were similar between both groups (data not shown). Whole mounts of tumors before they reached 800 mg weight are shown for both genotypes (Fig. 3). Lack of mammary fat tissue is evident in the C3(1)/T-Ag:A-ZIP/F-1 mice (Fig. 3*D*).

Serum levels of adipokines and growth-related hormones. To evaluate possible mechanisms that potentiate tumor development in A-ZIP/F-1 mice, the concentration of circulating hormones was examined. As expected, circulating fat-derived adipokines, such as leptin and resistin, were undetectable or very low in A-ZIP/F-1 mice (Fig. 4*A*). In contrast, growth hormone and IGF-I, which are known to promote tumor development (8, 19, 20), were statistically elevated in A-ZIP/F-1 mice, when compared with the levels of wild-type mice (Fig. 4*A*).

Cytokines and markers of inflammation. Because epidemiologic and experimental data correlate obesity and inflammation with increased cancer risk (22–25), we selected 14 inflammation-associated cytokines specifically reflecting Th1 and Th2 responses. A-ZIP/F-1 mice showed substantial increases in some cytokines typically associated with the Th2 response (IL-1β, IL-4, and IL-6), whereas there was no evidence of an increase in Th1 cytokines (TNF-α and INF-γ; Fig. 4*B*), indicating that A-ZIP/F-1 mice have an inflammatory condition. In addition, IL-12, MCP-1, and VEGF were also elevated. Cytokines not detectable in the serum were IL-2, TNF-α, GM-CSF, IL-10, IL-13, and IFN-γ.

"Kinetworks" phosphoprotein analysis. We examined mouse skin phosphoproteins at week 20 of the two-stage carcinogenesis

Table 2. Tumor types in C3(1)/T-Ag and C3(1)/T-Ag:A-ZIP/F-1 mice

	C3(1)/T-Ag	C3(1)/T-Ag:A-ZIP/F-1
No. of left thoracic glands examined	13	13
Adenomyoepithelioma	2	3
Carcinoma	1	4
Carcinoma, glandular	7	4
Carcinoma, adenosquamous	0	1
Carcinoma, papillary	0	1
Carcinoma, solid	0	2
Total	10	15

NOTE: Sixty-four mammary glands for C3(1)/T-Ag and 55 mammary glands for C3(1)/T-Ag:A-ZIP/F-1 were examined. C3(1)/T-Ag:A-ZIP/F-1 transgenic mice have a wider spectrum of carcinoma types and more numerous tumors compared with C3(1)/T-Ag mice.

experiment using the Kinexus phosphor-antibody screening platform. Duplicate samples from wild-type and A-ZIP/F-1 mice were tested using three different phospho-antibody screening panels (KPSS-1.3, KPSS-11.0, and KPSS-12.0). Figure 5 shows a representative immunoblot for a wild-type mouse and an A-ZIP/F-1 mouse for panel KPSS-1.3. Figure 5*B* shows the relative change, as a ratio, in the activity of the phosphoproteins in the skin of A-ZIP/F-1 mice compared with wild-type mice. Table 3 shows the relative change in selected protein kinases in the skin of A-ZIP/F-1 mice compared with wild-type mice from three different Kinexus phospho-antibody screening panels (KPSS). Data from the three different panels show an increase in the levels of phosphoproteins in the skin of A-ZIP/F-1 mice relative to wild-type mice. The increase in the kinase activity included proteins in two major pathways involved in survival and mitogenesis: the phosphatidylinositol 3-kinase (PI3K) pathway (downstream targets Akt, GSK3α, and PRAS40) and the Ras pathway (downstream targets MEK1/2 and ERK1/2). Furthermore, the upstream activators of both pathways (protein tyrosine kinases, such as ErbB2) were also activated. These data suggest that a mitogenic axis in the fatless A-ZIP/F-1 mice is activated at both a systemic level, as indicated by elevated circulating growth factors, and a tissue and cellular level, as indicated by activation of the protein tyrosine kinase signaling pathways.

Discussion

Epidemiologic studies show that obesity significantly increases the risk for many cancers, such as colorectal cancer and breast cancer (26, 27). Adipose tissue may enhance tumorigenesis in obese individuals through the excess production of cancer-associated adipokines (28, 29), the induction of insulin resistance, which can lead to type 2 diabetes, and/or the promotion of inflammation. The relative role of each of these underlying factors in the development of cancer is not clear. To separate the roles of obesity and fat tissue from insulin resistance and inflammation on carcinogenesis, we used the A-ZIP/F-1 mice, which lack white adipose tissue and produce undetectable levels of adipokines. These fatless mice are profoundly diabetic and display an inflammatory state. In the present study, we show that the

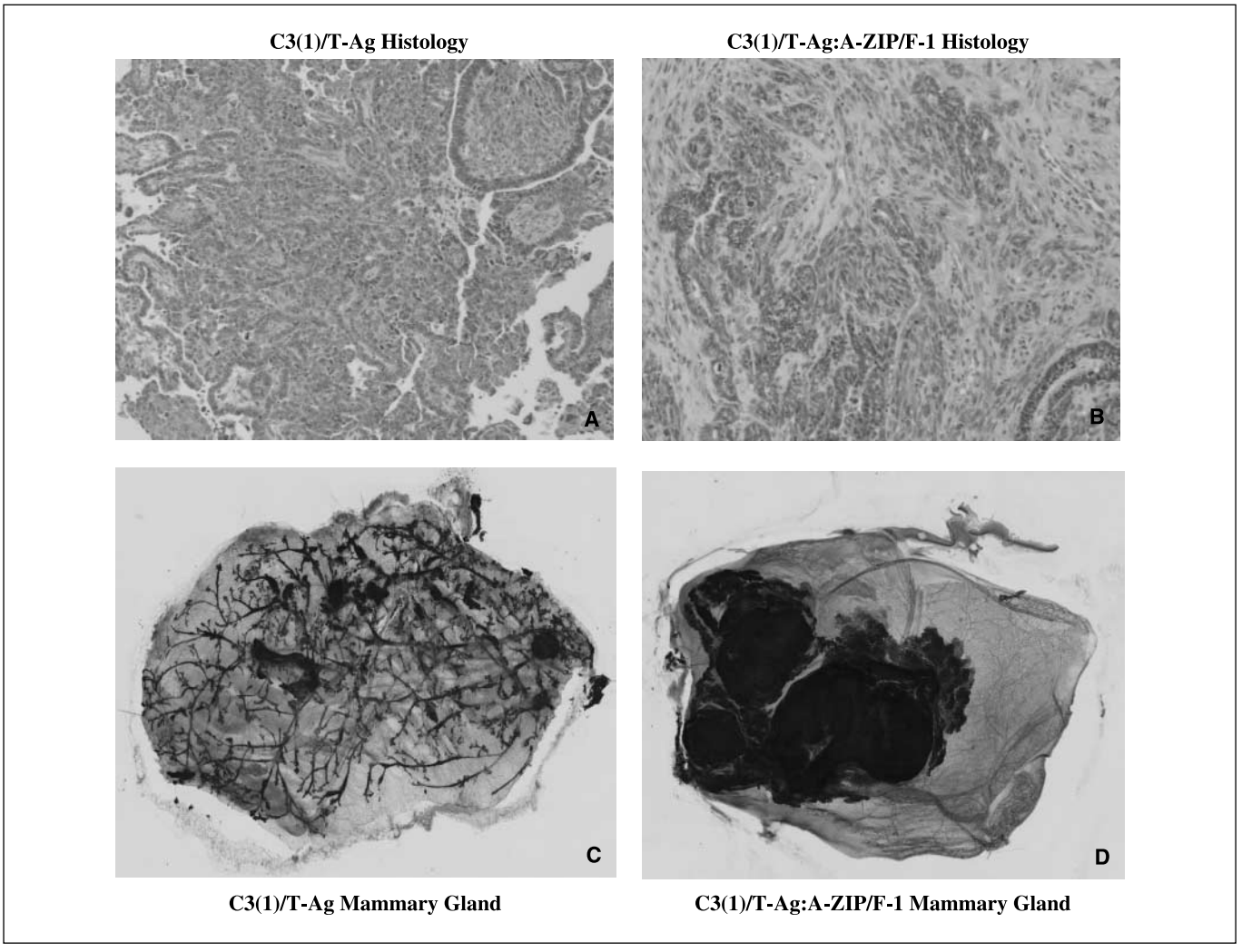

Figure 3. Mammary tumors from C3(1)/T-Ag and C3(1)/T-Ag:A-ZIP/F-1 genotypes. *A,* histology in the C3(1)/T-Ag tumors shows anaplastic carcinomas with a mixture of architectures: glandular, solid, and papillary. *B,* C3(1)/T-Ag:A-ZIP/F-1 tumors have similar morphology with additional stromal component and lack of fat tissue. *C,* whole mounts of C3(1)/T-Ag mammary glands with multiple small tumors. *D,* whole mounts of C3(1)/T-Ag:A-ZIP/F-1 mammary gland with tumors. Note large dilated mammary ducts and absence of terminal branching and fat cells. Female mice were 135 days old when the tumors and whole mammary glands were collected.

A-ZIP/F-1 mice have enhanced susceptibility to chemically-induced skin papillomas and genetically-induced mammary tumors. Two formal possibilities arise from the observation that the fatless A-ZIP/F-1 mice develop more tumors. One possibility is that adipose tissue and the secreted adipokines inhibit cancer growth. Experimentally, conflicting results have been observed. Most of these studies indicate that, whereas leptin potentiates the growth of cancer cells, adiponectin seems to have an opposite effect (28, 29). However, this possibility is not supported by the epidemiologic data showing a positive correlation between obesity and cancer. The second possibility is that other aspects of A-ZIP/F-1 mouse physiology, such as insulin resistance and/or inflammation, promote cancer growth.

There is ample epidemiologic and experimental evidence suggesting that physiologic alterations associated with type 2 diabetes, such as elevated levels of insulin, IGF-I, and growth hormone, can promote tumor formation (11, 19, 20) and an increase in insulin receptor expression is reported in human breast cancer cells (30, 31). Furthermore, epidemiologic data indicate that individuals taking supplemental insulin are more susceptible to colorectal cancer (32). Elevated glucose is also a risk factor for colon cancer (33). In addition, evidence supports the role of IGF-I and growth hormone in promoting cancer (8, 34–36). IGF-I- and growth-hormone–deficient mice are less susceptible to cancer, and supplementation with IGF-I and growth hormone increases their susceptibility (19, 20, 36). Growth hormone increases IGF-I levels in the liver (37), which may explain why IGF-I levels are elevated in the A-ZIP/F-1 mice. A-ZIP/F-1 mice have significantly elevated levels of all four of these risk factors: glucose, insulin, IGF-I, and growth hormone.

Examination of multiple phosphorylated proteins in the skin of both A-ZIP/F-1 and wild-type mice revealed several signaling pathways that are activated specifically in the A-ZIP/F-1 mice. Activation of the Ras pathway is expected in the two-step carcinogenesis model (38), but activation of the PI3K pathway (as reflected by phosphorylation of Akt and GSK3) was only evident in A-ZIP/F-1 mice. Cooperativity between Ras and PI3K pathways in cancer is well recognized (39). Thus, the A-ZIP/F-1 mouse has changes in circulating hormone and cytokine levels, as well as intracellular cell signaling pathways that are associated with insulin resistance, inflammation, mitogenesis, and increased cancer susceptibility.

Inflammation has also been implicated in obesity and tumorigenesis. There is growing support for the hypothesis that inflammation increases susceptibility to colorectal (40) and other cancers. For example, women taking over-the-counter nonsteroidal anti-inflammatory drugs had a 21% to 28% reduction in breast cancer incidence (41), providing further evidence that inflammation is a relevant target for cancer prevention. The A-ZIP/F-1 fatless mice have elevated levels of inflammatory cytokines IL-1β, IL-4, and IL-6, suggesting that these mice have a state of chronic inflammation. These cytokines are secreted by activated Th2 type

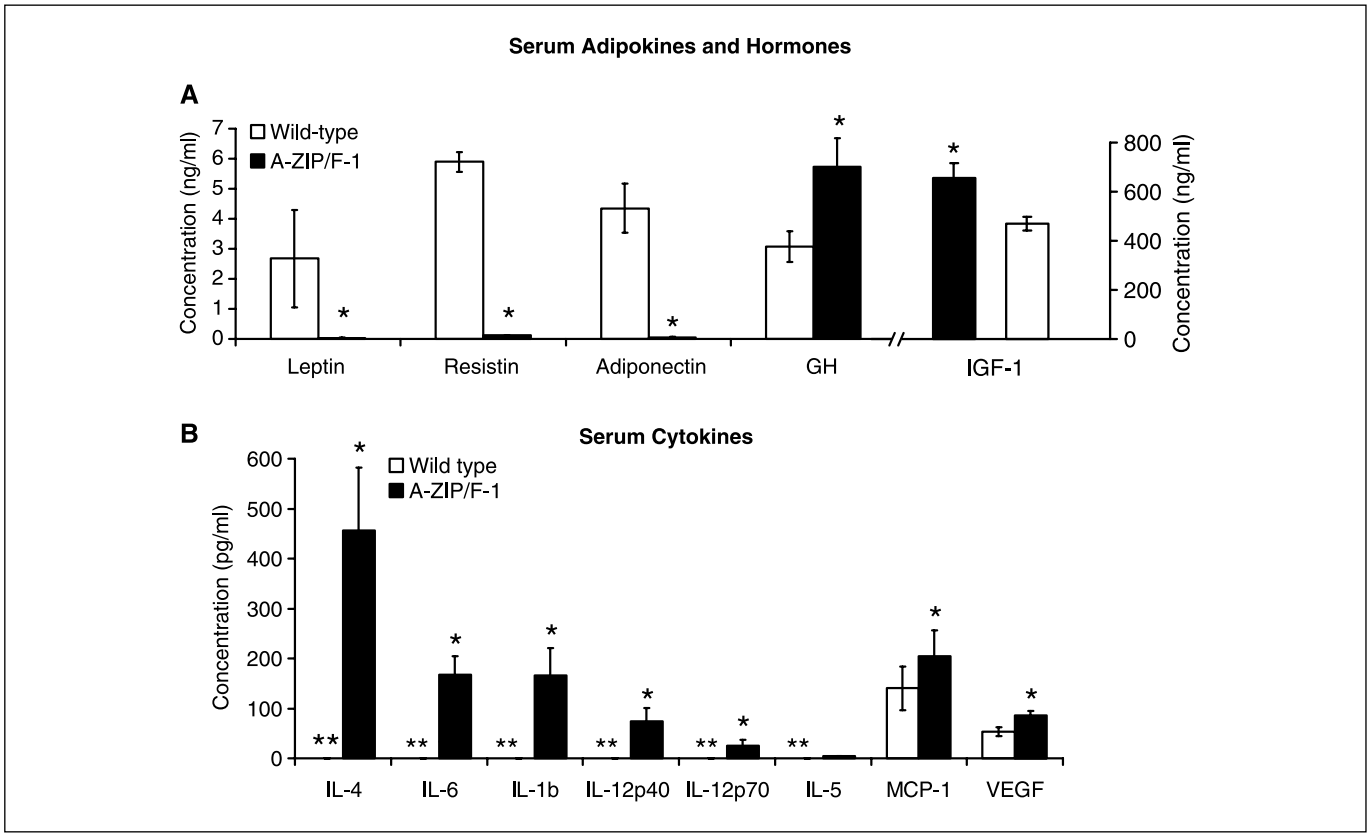

Figure 4. Serum levels of adipokines, hormones, and cytokines in A-ZIP/F-1 and wild-type female mice. *A,* leptin, resistin, and adiponectin (n = 12 for wild-type and n = 8 for A-ZIP/F-1 mice); growth hormone (*GH;* n = 19 for wild-type and n = 16 A-ZIP/F-1 mice) and IGF-I (n = 9 for wild-type and n = 17 for A-ZIP/F-1 mice). *Columns,* mean; *bars,* SE. *B,* serum cytokine levels in A-ZIP/F-1 and wild-type female mice. Fourteen-week-old mice were tested for serum cytokines (n = 5 for wild-type and n = 5 for A-ZIP/F-1 mice). *Columns,* mean; *bars,* SE. The lowest detectable level for all cytokines is 1 pg/mL, except for IL-5, which is 4 pg/mL. *, $P \leq 0.05$, statistical difference in adipokine, hormone, and cytokines levels between A-ZIP/F-1 and wild-type mice; **, cytokines were not detectable in wild-type mice.

Table 3. Phosphorylated proteins in the skin of A-ZIP/F-1 mice relative to wild-type mice

Panel KPSS-1.3	Ratio	Panel KPSS-11.0	Ratio	Panel KPSS-12.0	Ratio
JNK (SAPK)	0.36	Lyn (48)	0.38	Tau	0.50
MEK3/6	0.45	IR	0.44	eIF2a	0.56
CDK1/2	0.50	PKCb1/2	0.50	Rad17	0.67
RSK1/3	0.60	PKA/Ca/b	0.57	Pax2 (37)	0.67
B23 (NPM)	0.65	PKCg	0.75	Pax2 (36)	0.68
GSK3a	0.71	PKCh	1.28	Hsp27	0.69
p38a MAPK	0.72	PRK1	1.31	Dok2	1.22
PKCa	0.72	FAK-Y397	1.34	NMDA Nr2B	1.30
PKCd	0.72	PKCz/l	1.35	Cortactin	1.43
Jun (41)	1.20	GRK2	1.36	MARCKS	1.49
Src	1.24	PKCe	1.38	Erk1	1.51
Erk2	1.41	PKCb2	1.41	Erk2	2.20
Erk1	1.45	FAK-S843	1.58	Shc1	2.45
MEK1/2	1.46	PRK2	1.61	Adducin a	2.48
Smad1/5/8	1.53	PKCa	1.79	AcCoA carboxylase	2.76
PKR (73)	1.65	Erk1	1.81	PRAS40	2.99
STAT3	1.69	PKCa/b2	1.84	Cofilin 1	3.09
GSK3a	1.97	PKR (73)	2.26	eIF4G	3.97
Adducin a	2.24	Erk2	2.59		
PKBa (Akt1) [S473]	3.43	ErbB2	3.05		
PKBa (Akt1) [T308]	4.09	FAK-Y722	3.35		

NOTE: Phosphoproteins were measured using the Kinexus phospho-antibody screening system. Approximately 87 phosphoproteins were detected. The difference between A-ZIP/F-1 and wild-type mice is expressed as a fold change ratio. If the ratio is higher than 1, the kinase activity is increased in the A-ZIP/F-1 mice relative to the wild-type mice; if the ratio is below 1, it indicates that it is decreased.

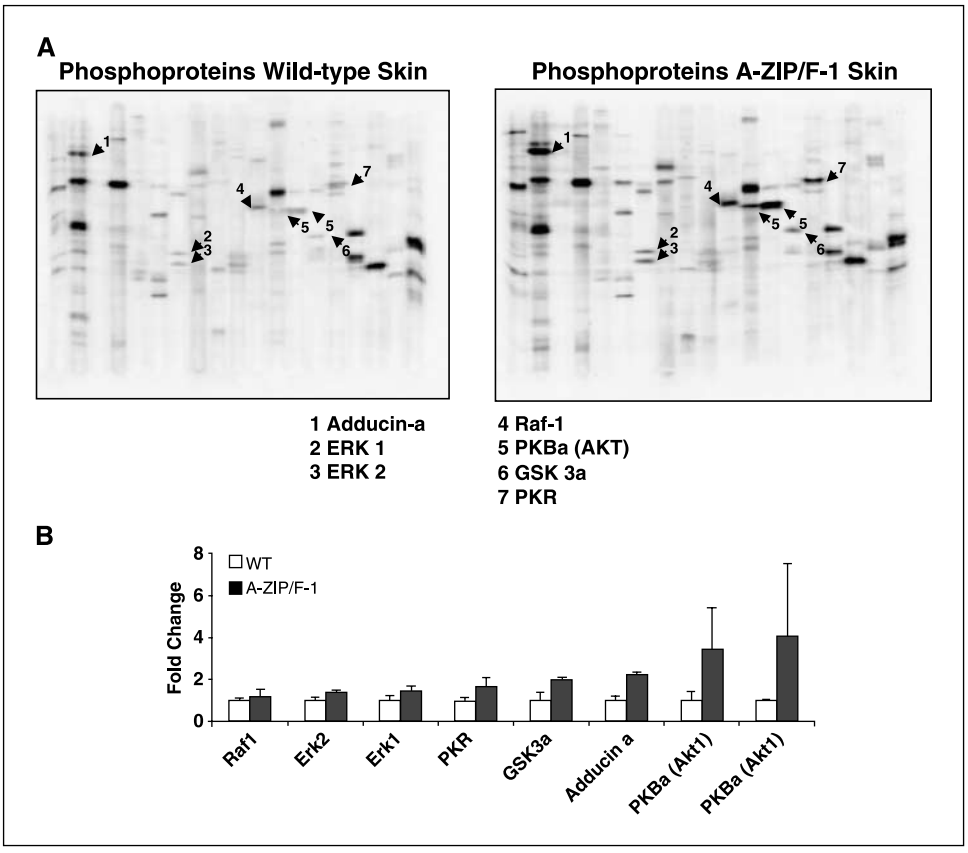

Figure 5. Phosphoproteins in the skin of A-ZIP/F-1 and wild-type mice during week 20 of the carcinogenesis protocol. *A,* an example of immunoblot for the Kinetworks phospho-site broad coverage pathway screen (KPSS-1.3) for skin extracts from wild-type and A-ZIP/F-1 mice. *B,* fold change for seven major phosphoproteins in A-ZIP/F-1 skin normalized for the level in the skin of wild-type mice.

(T helper) cells that are typically involved in the inflammatory response. In contrast, we did not find evidence of activation of the Th1 type T cells that are involved in the antitumor-cytotoxic-response (42), because TNF-α and IFN-γ were absent in A-ZIP/F-1 sera. It is not clear which of the inflammatory cytokines contributes to cancer susceptibility, but it is possible that IL-1 is critical for this response, because IL-1 regulates the expression of IL-6, IL-12, and VEGF, all of which are elevated in the A-ZIP/F-1 serum (43). The systemic effects of IL-1 may be further enhanced because fat is the major source of IL-1 receptor antagonist (IL-1rα; ref. 44).

In summary, the fatless A-ZIP/F-1 diabetic mice are more susceptible to tumor development despite their lack of white adipose tissue and adipokine production. This model does not allow one to examine whether adipose tissue from obese individuals contributes to increased carcinogenesis relative to a nonobese individual. The results presented here suggest that the increased risk of cancer in A-ZIP/F-1 mice may be related to a systemic promitogenic and proinflammatory environment rather than adiposity. We conclude that the A-ZIP/F-1 mouse provides a novel animal model that has the potential to further uncouple the relative roles of inflammation and insulin resistance/diabetes in carcinogenesis.

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