Inhibition of estrogen-independent mammary carcinogenesis by disruption of growth hormone signaling

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Clinical trials and laboratory-based studies indicate that the growth hormone/insulin-like growth factor-I axis may affect the development of breast cancer. The purpose of the present investigation was to develop a genetic model of mammary cancer to test the hypothesis that downregulation of GH signaling can substantially retard mammary cancer progression. We crossed the Laron mouse, in which the gene for the GH receptor/binding protein has been disrupted, with the C3(1)/TAg mouse, which develops estrogen receptor α negative mammary cancers. All mice used in our experiments were heterozygous for the large T antigen (TAg) and either homozygous wild-type for GHR (Ghr+/+) or null for GHR (Ghr−/−). Compared with the TAg/Ghr+/+ mice, the TAg/Ghr−/− mice showed delayed mammary cancer latency with significantly decreased multiplicity (9.8 ± 1.4 versus 3.2 ± 1.2) and volume (776.1 ± 284.4 versus 50.5 ± 8.9 mm3). Furthermore, the frequency of mammary hyperplasias was significantly reduced in the TAg/Ghr−/− mice (15.0 ± 1.7 versus 6.8 ± 1.7). To establish that these mammary cancers were estrogen-independent, 12-week-old TAg/Ghr−/− mice, which lack visible hyperplasia, were either ovariectomized (ovx) or sham operated (sham). Compared with the sham group, ovariectomy resulted in no difference in the frequency of mammary hyperplasia, mammary tumor latency, incidence, multiplicity or tumor size. Together, these data demonstrate that the disruption of GH signaling significantly retards TAg-driven mammary carcinogenesis, and suggest that disrupting GH signaling may be an effective strategy to inhibit the progression of estrogen-independent breast cancer.

Abbreviations: ERα, estrogen receptor alpha; GH, growth hormone; GHR/ BP, GH receptor/binding protein; IGF, insulin-like factor; MIN, mammary intraepithelial neoplasia; Tag, T antigen.

Introduction

Breast cancer remains the most common and second-most deadly form of cancer in American women (1), underscoring the continued need for new treatments. Traditional regimens include tumor excision, ovariectomy, radiation and chemotherapy with agents of broad toxicity. In recent years, signal transduction pathways have been elucidated and exploited in the development of novel substances for the prevention and therapy of breast cancer. Examples include small molecule drugs that downregulate estrogen signaling by either blocking estradiol biosynthesis or inhibiting its interaction with estrogen receptor alpha (ERα) (2). Another example is trastuzumab, which inhibits the growth of HER2 over-expressing tumors (3). While these treatments are much less toxic or invasive than older regimens, many patients’ tumors become independent of estrogen signaling or unresponsive to trastuzumab. Thus, there exists an urgent need to develop additional therapies that exploit different signaling pathways. Such drugs may not only be efficacious when used as single agents but may also prove to significantly enhance the efficacy of combination therapies by allowing the use of lower drug doses or less frequent administration protocols. Furthermore, combination therapies involving agents that act on diverse cellular targets are less likely to fail owing to tumor resistance.

One pathway that is a good candidate for anticancer drug development is the growth hormone/insulin-like factor (GH/IGF) axis. In vitro and in vivo studies of rodent and primate model systems illustrate that GH and IGF-I can induce mammary epithelial cell proliferation and differentiation while blocking apoptosis. Receptors for GH have been identified in mouse (4), rat (5), monkey (6) and human (7) mammary stroma. While the majority of receptors are found on stromal cells, mammary epithelial cells also express GH receptor (GHR) (8). The IGFs, their receptors and binding proteins are found during all stages of normal mammary gland development in all mammalian species examined to date (9). Kleinberg et al. (10) have shown that GH, acting through its receptors on mammary stromal cells, induces IGF-I that can act in paracrine fashion to stimulate parenchymal proliferation and differentiation (11).

The GH/IGF axis is important for the growth of advanced human breast cancers. Several independent laboratories have observed GHRs in human breast cancer cells (7,8,12,13). Pollak et al. (14) have reported that breast cancers derived from MCF-7 cells grow more slowly in mice homozygous for lit relative to control mice. Lit mice harbor a missense mutation resulting in loss of function of the pituitary GH-releasing hormone receptor and secondary suppression of GH and IGF-I. Schally et al. (15) have published studies recently that demonstrate an inhibitory effect of GH-releasing hormone (GHRH) antagonists on the growth of human mammary xenografts in nude mice. GHRH antagonists decreased IGF-I levels both in serum and tumors of treated
animals. Pollak et al. (16) have shown that mice expressing a bovine GH antagonist develop fewer mammary tumors when exposed to dimethylbenzanthracene compared with control mice. Friend et al. (17) have shown that the GH antagonist pegvisomant (Somavert®) inhibits the growth of human breast cancer cells grown as xenografts in immunodeficient mice.

The purpose of the present studies was to determine if disruption of GH signaling, effected by disrupting the GHR gene, could prevent estrogen-independent mammary cancers driven by the potent oncogene SV40.

Materials and methods

Animals

All studies involving animals were conducted in accordance with mandated standards of humane care as stipulated in the NIH Guide for the Care and Use of Laboratory Animals (18). Furthermore, the Institutional Animal Care and Use Committee of the University of Illinois at Chicago approved all experimental protocols involving animals before the initiation of any procedures. All the animals were bred at the Biologic Research Laboratories, University of Illinois at Chicago, were fed Teklad 8640 diet (Harlan Teklad, Madison, WI), given water ad libitum and housed in a temperature- and humidity-controlled environment with a regular light/dark illumination cycle (lights on at 06:00 and off at 19:00).

The 5’ flanking region of the C3(1) component of the rat prostate steroid binding protein was used by Green et al. (19) to target the expression of the SV40 large T-antigen (TAg) to the epithelium of both the mammary and prostate glands that, upon extensive characterization, have proven to be similar to both human breast (20,21) and prostate (22,23) cancer. Kopchick et al. (24) have used a homologous gene-targeting approach to disrupt the gene coding for both the GHR and the GH binding protein. The resulting knockout mouse closely resembles Laron syndrome (GH insensitivity syndrome), a disease of hereditary dwarfism due to defects in the GHR (25). Homozygous GH receptor/binding protein (GHR/BP) knockout mice (referred to here as Ghr−/−) display postnatal growth retardation, proportionate dwarfism and absence of the GHR and binding protein. Serum GH levels for Ghr−/− mice are greatly elevated compared with either Ghr+/+ or Ghr−/− mice (24). Serum IGF-I levels in Ghr−/− mice are decreased by ~90% compared with Ghr+/+ or Ghr−/− mice (26). No other abnormalities are evident in the homozygous or heterozygous knockout mice; their behavior is indistinguishable from that of their wild-type littermates, and lactation in Ghr−/− mice is adequate to feed their young.

To study the role of GH signaling in estrogen-independent mammary carcinogenesis, the C3(1) TAg mouse was crossed with the Laron mouse. The resulting progeny were genotyped and only mice heterozygous for TAg and homozygous for either wild-type GHR (Ghr+/+) or null for the GHR (Ghr−/−) were used. Genotyping was performed by PCR on DNA isolated by standard procedures (27) from tail snips. Genotyping for GHR was determined by PCR as described previously (26).

Animal procedures

Mice were palpated weekly beginning at 15 weeks of age and killed either at 30 weeks of age or upon development of a tumor >1 cm3. At the time of killing, individual tumor size and tumor location for each mouse was recorded. Tumor measurements were made with a digital caliper, and tumor volume was calculated (28) using the following formula: Tumor Volume = (π/6) × (larger diameter) × (smaller diameter)3. Whole mounts were prepared as described previously (29), stained in alum carmine and photographed in methyl salicylate. To study the hormone dependence of the mammary tumors, mice (TAg/Ghr−/−, n = 15) were ovariectomized before the appearance of mammary hyperplasias, but after the mammary epithelial tree filled the fat pad (12 weeks of age). Twelve-week-old mice were divided into ovariectomy (ovx) and control (sham, n = 45) groups. A subset of five mice were killed at the time of surgery and mammary tissue was harvested for analysis. Mice were palpated weekly for tumors and killed at 30 weeks of age or when tumors grew to ~1 cm3. Complete ovariectomy was confirmed at the time of killing by examining the surgery site for the presence of ovarian tissue and determining uterine weights. Mammary tissue free of palpable tumors was whole-mounted and examined for hyperplastic lesions using light microscopy.

Mammary gland whole mounts

Whole mounts were prepared to evaluate the impact of the GHR on mouse mammary gland morphology. The axillary mammary glands were excised, spread onto pre-labeled microscope slides, fixed in 10% neutral buffered formalin overnight, defatted in acetone and rehydrated in graded ethanol ending in water. The whole mounts were then stained with carmine (0.2% carmine, 0.5% aluminum potassium sulfate), washed in water and dehydrated in an ascending graded ethanol. Stained mammary gland whole mounts were stored and photographed in methyl salicylate (Sigma, St. Louis, MO).

The glands were examined through a dissecting microscope fitted with a reticle etched with a 1 mm2 grid. Mammary gland size was measured by calculating the whole axillary mammary gland with Meta Imaging series environment program. Mammary gland branch points and duct density were assessed by counting ductal branch points in at least 50 mm2, which was more than one quarter of the whole mammary gland area. Mammary hyperplasias were scored within a 100 mm2 area.

Histopathology, TAg and ERα immunohistochemistry

Formalin-fixed tissues were cut into 4 μm sections and placed on positively charged SuperFrost™ Plus slides (Fisher Scientific, Pittsburgh, PA). Endogenous peroxidase activity was quenched by incubating for 10 min in 3% H2O2 followed by several water rinses. To assess TAg expression, antigen retrieval was performed by heating sections in BD Retrievagen A (BD/Pharmingen, San Diego, CA) in a microwave oven for 10 min at 800 W. The sections were then covered for 20 min to allow them to cool to room temperature. Anti-SV40 TAg mouse monoclonal antibody (PharMingen, San Diego, CA) was used at a dilution of 1 : 50 for 1 h at room temperature. Bound antibody was observed by the ABC procedure and counter-stained with hematoxylin as described above. The procedure for ERα immunohistochemistry was the same as for SV40 described above with the following changes. Antigen retrieval was conducted in 0.01 M citrate buffer (pH 6.0). Anti-ERα (Novocasta, UK, diluted 1 : 40) was incubated for 2 h at room temperature. For histopathological analysis, sections were stained with hematoxylin and eosin and read by two independent pathologists who were blinded as to the origin of the specimens.

Serum IGF-I titers

Blood was drawn at the time of killing (30 weeks of age), allowed to clot centrifuged (800g, 4°C) and stored at −20°C until analysis for IGF-I by ELISA with OCTEA Rat/Mouse kit (Immunodiagnostic Systems, Fountain Hills, AZ) as described by the manufacturer.

Results

Mouse strain phenotype

Similar to GHR/BP−/− (Laron) mice (24), the TAg/Ghr−/− mice weighed significantly less than TAg/Ghr+/+ mice (Table 1). Serum IGF-I levels were 409 ± 29 ng/ml in TAg/Ghr+/+ mice but only 74 ± 22 ng/ml in TAg/Ghr−/− mice, which was significantly decreased by ~85% (P < 0.05).

These results are consistent with those seen in GHR/BP+/+ and GHR/BP−/− mice reported previously (24). Also, the mammary glands of the TAg/Ghr−/− mice were ~75% of the area of the TAg/Ghr+/+ animals. However, the numbers of

| Table 1. Selected physical characteristics of TAg/Ghr+/+ and TAg/Ghr−/− mice |
|-----------------------------|-----------------------------|-----------------------------|
| TAg/Ghr+/+                  | TAg/Ghr−/−                  |
| Body weight (g)             | 12.2 ± 0.6                  | 152.7 ± 11.0                |
| Mammary gland area (mm²)    | 5.2 ± 0.3                   | 5.3 ± 0.3                   |
| Ducts/cm²                   | 3.8 ± 0.2                   | 4.1 ± 0.2                   |
| Branch points/cm²           |                             |                             |

Values are expressed as means ± SEM. All mice were heterozygous for the TAg transgene and either homozygous for the GHR (TAg/Ghr+/+) or null for GHR (TAg/Ghr−/−). Animals were killed at 30 weeks of age or earlier if a tumor reached 1 cm3. While body weights and mammary gland area were greater in the Ghr+/+ mice relative to Ghr−/− mice, neither mammary ductal branch points nor the number of mammary ducts per unit area was significantly different between the two groups (n = 5; Student’s t-test, P > 0.05).
mammary ducts and ductal branch points per unit area of the dwarf mammary fat pad were not significantly different between TAg/Ghr<sup>+/+</sup> and TAg/Ghr<sup>−/−</sup> mice (Table I; Student’s t-test, \( P < 0.05 \)).

**Palpable mammary cancers**

Disruption of GH signaling substantially slowed mammary carcinogenesis driven by the SV40 transgene. At the time of killing, 50% of the TAg/Ghr<sup>+/+</sup> mice (\( n = 16 \)) developed tumors with a latency of 24 ± 1.9 weeks (median ± SE). In contrast, only 20% of the TAg/Ghr<sup>−/−</sup> mice (\( n = 25 \)) developed tumors with a median latency of 30 ± 2.2 weeks. Furthermore, the TAg/Ghr<sup>+/+</sup> group developed 9.75 ± 1.44 mammary tumors per mouse, while TAg/Ghr<sup>−/−</sup> mice bore only 3.2 ± 1.2 tumors per animal (Figure 1A; \( P < 0.05 \)). Also, the mammary tumors that developed in the TAg/Ghr<sup>−/−</sup> mice were significantly smaller than those that arose in the TAg/Ghr<sup>+/+</sup> group (Figure 1B and C; 51 ± 9 mm<sup>3</sup> versus 776 ± 284 mm<sup>3</sup>, respectively; \( P < 0.05 \)). Histologically, the tumors from the TAg/Ghr<sup>−/−</sup> mice were more differentiated and had a less aggressive morphology than the tumors from the TAg/Ghr<sup>+/+</sup> mice (Figure 1D and E). Mammary cancers of TAg/Ghr<sup>−/−</sup> mice were composed of differentiated structures with follicular or papillary organization. Areas of necrosis were few and small in size. Tumors cells showed limited heterogeneity and resembled human ductal carcinoma in situ (DCIS; Figure 1E). In contrast, the larger cancers of TAg/Ghr<sup>+/+</sup> harbored more necrotic areas and had a less differentiated morphology.

**Fig. 1.** Disruption of GHR inhibits mammary tumorigenesis. All mice (TAg/Ghr<sup>+/+</sup>; \( n = 25 \), TAg/Ghr<sup>−/−</sup>; \( n = 16 \)) were killed at 30 weeks of age. There were significantly fewer tumors per mouse in the TAg/Ghr<sup>−/−</sup> mice compared with the TAg/Ghr<sup>+/+</sup> mice (A: 9.8 ± 1.4 versus 3.2 ± 1.2; \( P < 0.05 \)). Furthermore, tumor volumes in the TAg/Ghr<sup>−/−</sup> mice were significantly smaller than those in TAg/Ghr<sup>+/+</sup> mice (B: 776.1 ± 284.4 versus 50.5 ± 8.9 mm<sup>3</sup>; \( P < 0.05 \)). Values are expressed as means ± SEM and were assessed by Student’s t-test. Panel C depicts a TAg/Ghr<sup>−/−</sup> mouse with no visible tumors and a TAg/Ghr<sup>+/+</sup> mouse bearing multiple, grossly apparent tumors. Panels D and E are photomicrographs of tumors from TAg/Ghr<sup>+/+</sup> and TAg/Ghr<sup>−/−</sup>, respectively. The less differentiated histopathology of the tumor from the TAg/Ghr<sup>−/−</sup> mouse (Figure 1D) compared with the tumor from the TAg/Ghr<sup>+/+</sup> mouse (Figure 1E) may be noted.
Tumor cells formed predominately solid structures lacking alveolar or papillary characteristics and resembled human DCIS or intraductal carcinomas (Figure 1D).

**Mammary hyperplasias**

Mammary glands either free of palpable mammary tumors or mammary tissue dissected free of tumors was examined for the presence of hyperplasias (Figure 2A and B). Mammary glands were fixed and stained as described above and examined through a dissecting microscope fitted with a micrometer reticle. Lesions were scored and categorized as either less than or greater than 1 mm² at longest dimension. One square centimeter of tissue was evaluated per mammary gland and the number of lesions per unit area was calculated. Fewer preneoplasias, both small and large, were observed in the TAg/Ghr−/− animals (Figure 2C).

Expression of TAg in TAg/Ghr<sup>+/+</sup> and TAg/Ghr<sup>−/−</sup> mice

An important potential problem with this project was that the lack of normal GH signaling in the TAg/Ghr<sup>−/−</sup> mice might downregulate TAg expression. This would be significant since a decrease in TAg expression in the TAg/Ghr<sup>−/−</sup> mice could explain their resistance to mammary carcinogenesis. To examine this question, mammary intraepithelial neoplasia (MIN) and tumor samples were analyzed by immunohistochemistry for TAg expression. As presented in Figure 3, TAg expression was similar in the TAg/Ghr<sup>−/−</sup> and TAg/Ghr<sup>+/+</sup> mice, suggesting that TAg expression is independent of the GH signaling pathway in the TAg/Ghr mouse.

**Ovarian independence of TAg/Ghr<sup>+/+</sup> mammary cancers**

Female TAg/Ghr<sup>+/+</sup> mice were ovariectomized (n = 15) or sham operated (n = 45) at 12 weeks of age, which was sufficient for the mammary tree to fill the fat pad but before the development of macroscopically (10× magnification) detectable mammary ductal hyperplasias. Careful inspection of all 10 mammary glands excised from the 12-week-old mice killed at the time of ovariectomy revealed no abnormal hyperplasias (data not shown). The remainder of the mice were palpated weekly for tumors and killed at 30 weeks of age or when tumors grew to ~1 cm³. The effectiveness of ovariectomy was confirmed at the time of killing by visual examination of the surgical site and by weighing the uteri. No ovarian tissue was observed at the time of killing, and ovariectomy resulted in significant loss of uterine mass relative to sham-operated controls (Table II; 23.7 ± 1.8 ovx versus 94.6 ± 4.9 sham). Nevertheless, mammary carcinogenesis as measured by the frequency of ductal hyperplasias, mammary tumor latency, multiplicity or volume was unaffected by removal of the ovaries and the associated lack of estrogens (Table II).

**Loss of ERα expression during mammary carcinogenesis in TAg/Ghr mice**

ERα expression was evaluated by immunohistochemistry in mammary glands and tumors obtained from both TAg/Ghr<sup>+/+</sup> and TAg/Ghr<sup>−/−</sup> mice. As shown in panels A and B of Figure 4, ERα expression was abundant in the normal mammary epithelia of both TAg/Ghr<sup>+/+</sup> and TAg/Ghr<sup>−/−</sup> mice. However, ERα expression was not observed in TAg/Ghr<sup>+/+</sup> or TAg/Ghr<sup>−/−</sup> mammary cancers.
In the current studies, we have crossed the C3(1)/TAg mouse, which develops estrogen-independent mammary cancers (19), with the GHR/BP knockout mouse (24) to develop a new strain, designated TAg/Ghr, that enabled us to study the role of GH signaling in mammary carcinogenesis. We designed the crosses such that the progeny used for the current studies were all heterozygous for the TAg transgene and either homozygous for normal GHR (TAg/Ghr\(^{++}\)) or null for GHR (TAg/Ghr\(^{-/-}\)). Mammary carcinogenesis was inhibited significantly as measured by tumor incidence, latency, multiplicity (Figure 1A) and volume (Figure 1B). Histologically, the tumors from the TAg/Ghr\(^{-/-}\) mice showed a more differentiated morphology associated with a less aggressive, more benign phenotype (Figure 4E and F). Also, the TAg/Ghr\(^{-/-}\) mice had fewer mammary hyperplasias (Figure 2), which histologically were MIN (Figure 3) and may represent precursors to mammary cancers, as has been established in other mouse strains (30).

**Discussion**

In the current studies, we have crossed the C3(1)/TAg mouse, which develops estrogen-independent mammary cancers (19), with the GHR/BP knockout mouse (24) to develop a new strain, designated TAg/Ghr, that enabled us to study the role of GH signaling in mammary carcinogenesis. We designed the crosses such that the progeny used for the current studies were all heterozygous for the TAg transgene and either homozygous for normal GHR (TAg/Ghr\(^{++}\)) or null for GHR (TAg/Ghr\(^{-/-}\)). Mammary carcinogenesis was inhibited significantly as measured by tumor incidence, latency, multiplicity (Figure 1A) and volume (Figure 1B). Histologically, the tumors from the TAg/Ghr\(^{-/-}\) mice showed a more differentiated morphology associated with a less aggressive, more benign phenotype (Figure 4E and F). Also, the TAg/Ghr\(^{-/-}\) mice had fewer mammary hyperplasias (Figure 2), which histologically were MIN (Figure 3) and may represent precursors to mammary cancers, as has been established in other mouse strains (30).

Since the mammary hyperplasias and tumors of the TAg/Ghr\(^{++}\) mice grew equally well in ovariectomized mice as in sham-operated animals (Table II) and these hyperplasias and tumors lacked ER\(_{\alpha}\) expression (Figure 4), we conclude that these neoplasias are estrogen-independent. Yoshidome et al. (31) have previously reported the time course for the loss of ER\(_{\alpha}\) expression during mammary carcinogenesis in the C3(1)/TAg mouse. Expression of ER\(_{\alpha}\) was detectable by 3 weeks of age and abundant by 8 weeks. However, at 14 weeks of age when high-grade MIN are observed and ER\(_{\alpha}\) was detected only in the basal layer of the mammary duct, and by 20 weeks of age, ER\(_{\alpha}\) expression was undetectable.

**Table II. Mammary carcinogenesis in the TAg/Ghr\(^{++}\) mouse is ovarian-independent**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Ovx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine weight (mg)</td>
<td>94.6 ± 4.9</td>
<td>23.7 ± 1.8</td>
</tr>
<tr>
<td>Hyperplasias per square centimeter</td>
<td>11.4 ± 1.3</td>
<td>11.5 ± 1.1</td>
</tr>
<tr>
<td>Tumor latency (weeks)</td>
<td>22.1 ± 0.3</td>
<td>22.6 ± 0.5</td>
</tr>
<tr>
<td>Tumor multiplicity</td>
<td>11.2 ± 1.3</td>
<td>9.5 ± 0.6</td>
</tr>
<tr>
<td>Tumor volume (mm(^3))</td>
<td>592 ± 64.7</td>
<td>484 ± 88</td>
</tr>
</tbody>
</table>

Twelve-week-old TAg/Ghr\(^{++}\) mice were sham-operated (sham; \(n = 45\)) or ovariectomized (ovx, \(n = 15\)) and killed at 30 weeks of age. While uterine weight was significantly decreased by ovariectomy, confirming the success of the surgery, no differences were evident between ovx and sham groups in the frequency of preneoplasias or tumor latency, multiplicity or volume (Student’s \(t\)-test at 5% level of significance). Values represent means ± SEM.

TAg/Ghr\(^{-/-}\) mice in either MIN (Figure 4C and D) or mammary cancers (Figure 4E and F).
powerful oncogene (TAg) that disrupts p53, which is one of the most commonly mutated genes in human cancers (32). This finding may have important translational significance for patients with ERα negative tumors.

The reason that signaling through the GHR is required for estrogen-independent mammary cancers is not known. However, a growing body of literature indicates that stimulation of the estrogen signaling pathway can down-regulate or antagonize signaling by the GH/IGF axis (33). For example, estrogens have for decades been used clinically to suppress long bone growth in adolescent girls predicted to grow taller than 183 cm (34). In high doses, estrogens counteract GH by stimulating cartilage maturation without increasing growth, thus inducing closure of epiphyseal plates.

**Fig. 4.** Expression of ERα is not modified by GHR and appears to be lost during mammary tumor progression. Formalin-fixed, paraffin-embedded sections of mammary glands and mammary tumors excised from TAg/Ghr+/+ mice or TAg/Ghr−− mice were analyzed by immunohistochemistry for ERα expression. (A and B) Mammary gland derived at 10 weeks of age; no MIN lesions were observed, and the expression of ERα was clearly detectable in normal-looking epithelium cells. It may be noted that the expression of ERα in the mammary gland derived from the TAg/Ghr+/+ mice (A) is similar to that observed in the mammary gland derived from the TAg/Ghr−− mice (B). (C and D) Mammary glands derived at the time of killing; high-grade MIN are observed, whereas ERα was undetectable. It may be noted that mammary glands from both TAg/Ghr+/+ mice type (C) and TAg/Ghr−− mice (D) did not express ERα. (E and F) Mammary tumors derived at the time of killing. It may be noted that ERα expression was undetectable in both TAg/Ghr+/+ mice (E) and TAg/Ghr−− mice (F). Original magnification: 40x.
in long bone. Estrogens can also decrease serum IGF-I (35), IGFBP-3 and the acid-labile subunit (36), which is believed to explain how estrogen treatments can ameliorate the symptoms of acromegaly (37). In GH-deficient patients, men are more responsive to GH replacement therapy than women (38), suggesting that estrogens can antagonize the effects of GH in many peripheral tissues. GH exerts its effects on target cells by binding the GHR and activating the JAK/STAT pathway (39). At the molecular level, estrogens can inhibit STAT5 tyrosine phosphorylation, DNA-binding (40) and activation of the β-casein promoter (41). Also, estrogen can inhibit GH-mediated phosphorylation of JAK2 by upregulating the expression of SOCS2, a key attenuator of GH signaling (42). Given the negative effect that estrogens have on GH signaling observed from the molecular level to the clinic, it is possible that when mammary cancers evolve from estrogen-dependent, ERα positive tumors to estrogen-independent, ERα negative tumors, GH/IGF-I signaling, freed from suppression by estrogen signaling, may become as critical to ERα negative tumor survival as estrogens are for the survival of ERα positive mammary tumors.

The new mouse strain developed here, TAg/Ghr, should prove useful for probing the role of GH signaling in mammary carcinogenesis. Each of the parent strains of the TAg/Ghr mouse has been thoroughly characterized and considered to be excellent models for the human. The GHR/BP knockout (Laron) mouse exhibits proportionate dwarfism, absence of the GHR and GHBP, decreased serum IGF-I and elevated serum GH levels, thus representing the Laron phenotype in humans (25). Mammary carcinogenesis in the C3(1)/Tag mouse progresses in a measured fashion through MIN, which resemble human DCIS, to mammary cancers similar in histology to infiltrating ductal carcinomas in human breast cancer (20). Mammary glands in the adult TAg/Ghr−/− mice had a similar number of branch points and density of ducts compared with TAg/Ghr+/+ mice (Table I), suggesting that, although the overall gland size was smaller, normal branching morphogenesis was not affected by the loss of the GHR. Also, the expression of TAg was not affected by the loss of GHR since TAg protein was clearly evident in both MIN and mammary cancers (Figure 3). Therefore, inhibition of mammary carcinogenesis in TAg/Ghr−/− mice is due to the inhibition of cancer progression of initiated cells. This is manifest by the lower frequency and size of both mammary hyperplasias (Figure 2) and mammary tumors (Figure 1).

An important question for future studies is to determine if the lack of GHR is solely or directly responsible for protection of the TAg/Ghr−/− mouse from mammary carcinogenesis or whether there are other factors, such as IGF-I, through which the GHR knockout prevents cancer. It is likely that low levels of IGF-I in the TAg/Ghr model contributes to the protection from carcinogenesis afforded by the GHR knockout. It is well established that IGF-I signaling plays an important role in carcinogenesis (43). In the GHR/BP knockout mouse, the lack of GHR results in a 90% reduction in serum titers of IGF-I while GH serum levels are elevated owing to the lack of a functional negative feedback system (24). We observed that IGF-I was also dramatically reduced (82%) in the TAg/Ghr−/− mouse. Therefore, mammary carcinogenesis is prevented in an environment where target cells are insensitive to GH and the blood is deficient in IGF-I. While local production of IGF-I in neither GHR/BP knockout nor TAg/Ghr mice has been evaluated, it is likely that production of IGF-I by the mammary gland is significantly decreased. Kleinberg et al. (11) have suggested that GH, acting through its receptors in mammary stroma (4), stimulate stromal IGF-I production, which in turn activates IGF-I receptors in the adjacent mammary epithelial cells. However, with GHR knocked out throughout the TAg/Ghr−/− mice, it is likely that the paracrine production of IGF-I within the mammary gland is also blocked.

Irrespective of whether disruption of GH signaling acts directly on tumor tissue, through modulation of the IGF system, or by both mechanisms, data presented here suggest that disrupting the GH pathway is an effective means of suppressing mammary carcinogenesis. Furthermore, since carcinogenesis proceeds in ovariectomized TAg/Ghr−/− mice to yield ERα negative mammary tumors (Table II, Figure 4), downregulation of GHR can prevent estrogen-independent mammary cancers. These results have important translational significance for patients bearing estrogen-independent cancers that are currently limited to highly toxic drug therapies. While a number of laboratories and pharmaceutical companies are working to develop inhibitors of IGF-I signaling (44), no drug has yet been approved. On the other hand, the FDA has approved the GH antagonist pegvisamont for daily, long-term use in the treatment of acromegaly (45). Since pegvisamont is well tolerated in patients, it may be appropriate for use as a second-line therapy in cancer patients to prevent a relapse of disease or in patients who must stop tamoxifen treatments owing to its 5-year regimen limit.

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