Carcinogenesis vol.23 no.6 pp.977–982, 2002

The growth hormone-deficient Spontaneous Dwarf rat is resistant to chemically induced mammary carcinogenesis

Steven M.Swanson[1,2,4] and Terry G.Unterman[3]

[1]Program for Collaborative Research in the Pharmaceutical Sciences and the Center for Pharmaceutical Biotechnology, [2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy and [3]Veteran's Administration Chicago Healthcare System (West Side Division), and Department of Medicine, Section of Endocrinology and Metabolism, College of Medicine, University of Illinois at Chicago, Chicago, IL 60612, USA

[4]To whom correspondence should be addressed
Email: swanson@uic.edu

Recent epidemiologic studies have suggested that the growth hormone (GH)/insulin-like growth factor I axis plays an important role in human breast cancer. The purpose of the present study was to evaluate the function of GH in rat mammary carcinogenesis, a model that closely recapitulates human breast cancer biology. The Spontaneous Dwarf rat (SDR) arose from the Sprague–Dawley rat and harbors a mutation in its GH gene yielding undetectable levels of a severely truncated protein not capable of binding to the GH receptor. When female rats of either strain were exposed to the direct-acting carcinogen *N*-methyl-*N*-nitrosourea, all wild-type rats ($n = 10$) developed multiple mammary cancers (5.3/rat). In contrast, SDR rats ($n = 15$) developed only three cancers (0.2/rat) and these were very small (<6 mm^3). In another experiment, SDRs were backcrossed with wild-type Sprague–Dawley rats and the progeny were exposed to the indirect-acting carcinogen 7,12-dimethylbenz[*a*]anthracene. Progeny that were either homo- or heterozygous for the wild-type GH gene developed ~4 mammary tumors/rat, respectively. In contrast, SDR progeny developed only 0.21 tumors/rat. Mammary glands of SDRs had substantially less alveolar development compared with wild-type, yet ductal branching was similar in the two strains. Infusion of rat GH to SDRs induced mammary epithelial cell proliferation and alveolar development similar to that of wild-type rats. Taken together, these results demonstrate an important role for GH in alveolar development in the virgin rat, and provide the first direct evidence that GH plays a critical role in mammary carcinogenesis.

Introduction

Breast cancer is the most common form of cancer and the second deadliest form of this disease among American women (1). Treatment regimens have historically included tumor excision, ovariectomy, radiation and chemotherapy with agents of broad toxicity. In recent decades, signal transduction pathways that regulate mammary epithelial cell proliferation, differentiation and apoptosis have been elucidated and novel agents have been developed to target these pathways. Examples include selective estrogen receptor modulators (SERMs) (2) and trastuzumab (Herceptin) (3), which target estrogen receptor and HER-2-mediated pathways, respectively. While these important drugs are effective in a portion of breast cancer patients, many tumors do not express the estrogen receptor (4) or do not overexpress HER-2 (5), excluding them as candidates for these therapies. Most importantly, clinical data demonstrate that a combinatorial approach to the treatment of cancer is superior to single-agent therapy (6). Clearly, additional therapeutic strategies that target different signal transduction pathways involved in the development and progression of breast cancer are needed.

One such pathway involves the growth hormone (GH)/insulin-like growth factor-I (IGF-I) 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. An early full-term pregnancy is protective against mammary cancer in both rats and humans. We have reported previously (7) that GH serum titers are significantly reduced in parous rats relative to age-matched virgins suggesting that lower circulating GH may contribute to the protective effect of pregnancy against rat mammary cancer. IGFs are believed to be important mediators of GH action. GH induces the mammary stroma to synthesize IGF-I, which can stimulate proliferation in the adjacent mammary epithelium in a paracrine manner (8). The IGFs are unique among mammary growth factors in that they also may serve endocrine functions. GH stimulates IGF-I production by the liver, thereby elevating circulating IGF levels. This endocrine aspect of IGF physiology has facilitated epidemiologic studies on the relationship of GH and IGFs to cancer risk. Clinical studies, including some prospective studies, indicate that elevated circulating IGF-I or IGF-I/IGFBP-3 ratio confer an increase in risk for the development of lung, prostate and breast cancers (9). The effects of the GH/IGF-I axis on mammary epithelial cell proliferation, differentiation and apoptosis as well as its association with human cancer risk implicate this pathway as a potential candidate for the targeting of novel therapeutic strategies.

A key step in this process is the identification of appropriate animal models for evaluating the role of the GH/IGF-I axis in the development of mammary cancer. The Spontaneous Dwarf rat (SDR) was first identified in a colony of Sprague–Dawley rats in 1977 (10,11) and harbors an autosomal recessive mutation (gene symbol *dr*) in its GH gene (12). The error is a point mutation in the consensus sequence of the 3′ splice site of the third intron, resulting in premature translational termination. Somatotropes are scarce in pituitaries of SDRs as judged by histological examination, and no GH is detectable by immunohistochemistry (13,14). However, corticotropes and mammotropes are present and morphologically normal. Serum titers of IGF-I are ~10% of that observed in wild-type rats (15). Both male and female SDRs are fertile. At 25 weeks of

Abbreviations: BrdU, bromodeoxyuridine; DMBA, 7,12-dimethylbenz[*a*]anthracene; IGF, insulin-like growth factor; MNU, *N*-methyl-*N*-nitrosourea; SDR, the Spontaneous Dwarf rat.

age, body weights of the females are only ~45% that of age-matched wild-type Sprague–Dawleys (10). Nevertheless, the relative organ weights of the SDR are similar to that observed in the wild-type rats (10). In short, the SDR is an excellent model of isolated GH deficiency.

The exposure of rats to the alkylating agent *N*-methyl-*N*-nitrosourea (MNU) or the polycyclic aromatic hydrocarbon 7,12-dimethylbenz[*a*]anthracene (DMBA), which work by distinct molecular mechanisms, are the best-characterized and most widely used models of human breast cancer (16). Rats, particularly Sprague–Dawley rats, exposed to these carcinogens develop mammary cancers that closely resemble human breast cancer in many important ways such as histopathology, hormone dependence and response to chemotherapeutic agents. The generation of the SDR from the Sprague–Dawley presents a unique opportunity to assess the function of GH in mammary carcinogenesis using an animal model of isolated GH deficiency.

Materials and methods

Mammary carcinogenesis

All experiments involving animals were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (17) and under a protocol approved by the Institutional Animal Care and Use Committee of the University of Illinois at Chicago. SDR were bred at the Biologic Resources Laboratory, University of Illinois at Chicago. Wild-type Sprague–Dawley rats were purchased from Harlan Sprague–Dawley, Indianapolis, IN. All rats were fed a Teklad 8640 diet (Harlan Teklad, Madison, WI) and 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). In the first experiment, the SDR was compared with the Sprague–Dawley rat for susceptibility to MNU-induced mammary carcinogenesis. Seven-week-old rats ($n = 10$ Sprague–Dawley, $n = 15$ SDR) were given a single intraperitoneal injection of MNU (50 mg/kg, Ash-Stevens, Detroit, MI) dissolved in 0.85% saline acidified with acetic acid. Once each week thereafter, the rats were weighed and palpated for the presence of mammary tumors. Six months after carcinogen administration, all rats were killed and the mammary tumors were excised. A section of each tumor was fixed in 10% neutral buffered formalin and processed for histologic classification (18). Tumor volumes were calculated (19) using the formula: $V = (\pi/6)\times(\text{larger diameter})\times(\text{smaller diameter})^2$. Fisher's exact test was used to analyse mammary cancer incidence rates and the Armitage test (20) was used to evaluate mammary cancer multiplicity.

The SDR arose in 1977 and has since been bred apart from its founder strain, the Sprague–Dawley. To control for factors resulting from genetic drift, a Sprague–Dawley rat was purchased from Harlan Sprague–Dawley to back cross with the SDR. The progeny of these matings were genotyped using a PCR/RFLP method modified from that of Nogami *et al*. (21). A 408 bp segment of the rat GH gene was amplified by PCR using 5′-ttccctgaggctgaggtaac-3′ and 5′-ctccagaacctagagaaaggc-3′ as forward and reverse primers, respectively. This amplified sequence contains the G to A mutation that is located in the splicing consensus of the 3′ end of the third intron of the GH gene in SDR (12). The mutation lies in one of the three recognition sequences of the *Sau*I restriction enzyme in the amplified fragment. *Sau*I cuts the wild-type allele (DR) in three places yielding four restriction fragments (192, 145, 66 and 5 bp). Digestion of DNA from heterozygotes yields 258, 192, 145, 66 and 5 bp fragments. Digestion of homozygous dr rats produces only three fragments (258, 145 and 5 bp). The restriction fragments of an overnight digestion are separated on an agarose gel and can be used to distinguish DR/DR, DR/dr and dr/dr animals.

Rats were divided into three groups based on their genotype: homozygous wild-type (DR/DR, $n = 15$), heterozygotes (DR/dr, $n = 15$) and SDR (homozygous mutant dr/dr, $n = 14$). At 7 weeks of age, each rat received a single treatment with DMBA (20 mg/kg body wt) by gavage. This carcinogen was chosen as in rats it induces mammary cancers that have been thoroughly characterized (18) and its mechanism of action is distinct from that of MNU. While MNU is a direct-acting DNA methylating agent, DMBA induces bulky adducts at different DNA sequences after metabolic activation by P450 enzymes. Beginning 3 weeks after DMBA administration, each rat was weighed and palpated for mammary tumors weekly.

Infusion with recombinant rat GH

The assumption made in these experiments is that the *dr* mutation is the only genetic difference between the SDR and the Sprague–Dawley that significantly impacts mammary gland biology. If this were true, supplementation of SDRs with physiologic levels of GH should restore the wild phenotype. To test this hypothesis at the level of gland morphology, 7-week-old female SDR littermates were treated with GH via mini-osmotic pumps (model 2001, Alzet Osmotic Pumps, Durect, Cupertino, CA) implanted subcutaneously under ketamine/xylazine anesthesia. Recombinant rat GH was provided by Dr A.F.Parlow, Director, National Hormone and Peptide Program, NIDDK. Mini-osmotic pumps were filled with GH dissolved in 10 mM NaOH or aqueous NaOH vehicle only. Each rat received one pump for 7 days. Each pump released the GH solution (10 μg/μl) at a rate of 1 μl/h. This dose of GH is known to stimulate growth and to provide physiologic serum titers of IGF-I in SDRs (22).

Mammary gland whole mounts

Whole mounts were prepared to evaluate the impact of the *dr* mutation on rat mammary morphology. The numbers four and five (abdominal/inguinal) mammary glands from each rat were excised and spread onto pre-labeled microscope slides (75×50×1 mm). Mammary gland spreads were fixed overnight in alcoholic formalin (Pen-Fix, Richard-Allan Scientific, Kalamazoo, MI), defatted in acetone and rehydrated in a graded series of ethanol ending in water. The whole mounts were then stained in alum carmine for 1–4 days. After staining, the whole mounts were washed in water and dehydrated in an ascending graded series of ethanol and cleared in toluene. Stained mammary gland whole mounts were stored and photographed in methyl salicylate (23).

Mammary gland branching was assessed by counting ductal branch points in a 20 mm^2 swath from the nipple, through the lymph node to the leading edge of parenchymal penetration into the fat pad. This area was chosen to include the several mammary gland zones that are reported to vary with respect to their level of differentiation in the developing rat (24). The glands were examined through a dissecting microscope fitted with a reticle etched with a grid of 1 mm squares. To compare the density of terminal end buds, the grid was employed to count these structures at the growing front of the mammary glands where the parenchyma invades the bare fat pad. To estimate mammary gland area, the width was taken as the distance from the nipple to the furthest end bud and the length was measured along a line bisecting the lymph nodes.

Quantification of cell proliferation

To quantify cell proliferation, bromodeoxyuridine (BrdU) uptake by the nuclei was determined using an immunohistochemical method based on that of McGinley *et al.* (25). This technique involves the fixation of tissues in methacarn and yields quantitative epitope recovery and labeling indices similar to those observed in frozen tissue sections. Rats received an intraperitoneal injection of BrdU (50 mg/kg body wt) dissolved in phosphate-buffered saline 2 h prior to death. Pieces (~1 cm^2) of each animal's right thoracic (#3) mammary gland were fixed overnight in methacarn (methanol:chloroform:glacial acetic acid, 6:3:1). The following day, the tissues were processed for immunohistochemical analysis of BrdU, as described (25).

Results

SDRs (63.8 ± 1.9 g) were less than one-half the size of the wild-type Sprague–Dawleys (135 ± 2.0 g) at 50 days of age, consistent with previous studies (10). There was concern that the SDRs might not survive the MNU exposure. However, the animals tolerated the carcinogen well and there were no fatalities in either group. No acute toxicity was grossly evident except for a temporary deceleration of body weight gain in both rat strains that lasted <1 week. Within 1 month after carcinogen, the body weights of MNU-exposed SDRs (75.7 ± 3.6 g) were not significantly different from untreated age-matched SDRs (76.5 ± 4.3 g, $P > 0.05$, two-tailed Student's *t*-test). All animals survived until the end of the experiment 6 months after MNU exposure (~230 days of age).

As illustrated in Figure 1, wild-type Sprague–Dawley rats are highly susceptible to MNU. All rats developed multiple palpable mammary cancers (53 total) that were primarily papillary carcinomas. The number of mammary tumors per rat at termination was 5.30 ± 0.83 (mean ± SEM, Table I). In stark contrast, the SDR group developed only four tumors

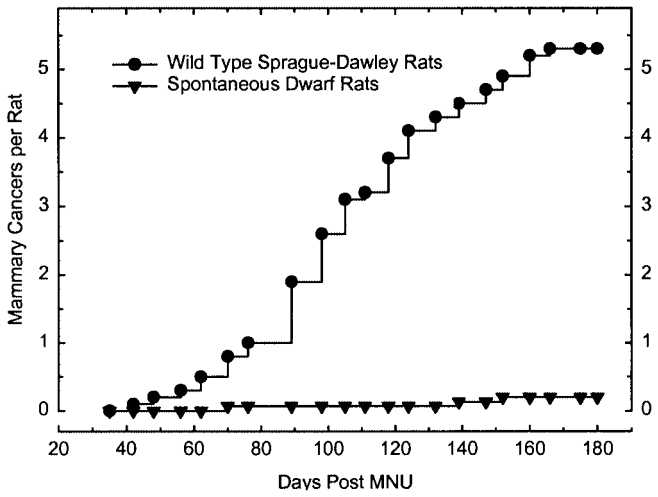

Fig. 1. Latency and multiplicity of mammary cancers induced by MNU in SDR ($n = 15$) or wild-type Sprague–Dawley rats ($n = 10$). Rats were exposed to a single intraperitoneal injection of MNU at 7 weeks of age. The control Sprague–Dawley rats developed 53 tumors, all of which were carcinomas. The SDR developed three small (<2 mm diameter) mammary cancers.

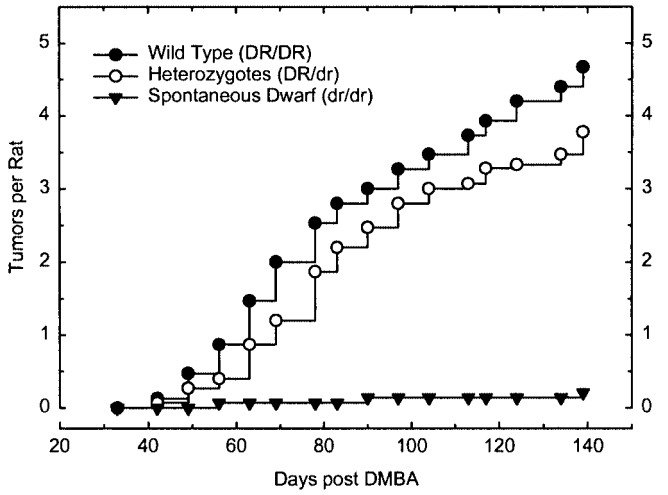

Fig. 2. Latency and multiplicity of mammary tumors induced by DMBA in cohort of rats descendent from back crossing SDR with a wild-type Sprague–Dawley rat. Rats were exposed to a single gavage of DMBA (20 mg/kg) at 7 weeks of age.

Table I. Mammary carcinogenesis summary for Sprague–Dawley and SDR rats exposed to MNU

Mammary cancer parameter	Sprague–Dawley	SDR
Number of rats	10	15
Total number of cancers	53	3
Multiplicity (cancers/rat)	5.30 ± 0.83	0.20 ± 0.11[a]
Incidence (%)	100	20[b]
Median latency (weeks)	15	22

[a]Significantly different from wild-type Sprague–Dawley ($P < 0.05$, Armitage's test for trend in proportions).
[b]Significantly different from wild-type Sprague–Dawley ($P < 0.001$, Fisher's exact test).

Table II. Mammary tumorigenesis summary for a cohort of DMBA-exposed rats generated by back crossing the SDR with the wild-type Sprague–Dawley rat

Mammary tumor parameter	DR/DR	DR/dr	dr/dr (SDR)
Number of rats	15	14	14
Total number of tumors	70	56	3
Multiplicity (tumors/rat)	4.67 ± 0.73	3.79 ± 0.60[a]	0.21 ± 0.11[b]
Incidence (%)	93	100	21
Median latency (weeks)	11	11	13

[a]Significantly different at 5% level from SDR but not controls (Armitage's test for trend in proportions).
[b]Significantly different from either wild-type or heterozygous groups ($P < 0.05$, Fisher's exact test).

(one benign and three malignant). The largest tumor was a fibroadenoma 900 mm^3 in volume, which is common for mammary tumors in MNU-exposed rats. Of the three cancers, one was a comedo carcinoma, and the others were papillary carcinomas. The cancers were quite small: 5.6, 2.1 and 1.4 mm^3, respectively. The latencies were 137, 152 and 160 days. The median latency in the control group was 105 days (Table I). Necropsy at termination revealed no grossly observable lesions in any other major organ system. However, the numbers four and five mammary glands of each SDR were mounted to check for preneoplastic lesions. Of the 30 glands inspected, only two hyperplasias were observed.

In experiment 2, a cohort of rats was generated by backcrossing the SDR with the Sprague–Dawley rat. Progeny from these crosses were grouped according to their genotype and exposed to DMBA as described in the Materials and methods. Weights for homozygous wild-type (Sprague–Dawley) and heterozygote animals were not significantly different at any age (data not shown), indicating that GH is sufficient in heterozygotes to maintain normal growth activity. One animal heterozygous for wild-type GH gene (DR/dr) died midway through the study and was not included in the analyses. As illustrated in Figure 2, rats homozygous (DR/DR) or heterozygous (DR/dr) for wild-type GH protein were highly susceptible to DMBA-induced mammary tumorigenesis. The homozygous wild-type rats (DR/DR) developed 4.67 ± 0.73 mammary tumors/rat and the heterozygotes developed 3.79±0.60. The SDRs (dr/dr), in contrast, developed only three small tumors (0.21 ± 0.11 tumors/rat, Table II).

Feldman *et al.* (26) have reported that treatment of hypophysectomized and ovariectomized rats with rat GH and estrogens can stimulate mammary gland development as judged by increased numbers of terminal end buds or alveolar structures. However, hypophysectomy drastically reduces the serum titers of several other mammotropic hormones such as glucocorticoids, thyroxin and prolactin in addition to GH. As the SDR is a model of isolated GH deficiency, it is better suited for studies on the role of this hormone in mammary gland development. Evaluation of mammary gland whole mounts prepared from 8-week-old wild-type Sprague–Dawley rats or age-matched SDRs revealed substantial morphologic differences between the two strains. SDR mammary glands were about half the size of glands from Sprague–Dawley controls (Figure 3A and B, Table III). The degree of branching as determined by branch point analysis was not significantly different between SDR and wild-type Sprague–Dawley rats (Table III). In wild-type Sprague–Dawley whole mounts, alveolar structures are observed sprouting from ducts throughout the gland from the primary duct to the vicinity of the end buds (Figure 3A and D). In marked contrast, whole mounts of SDRs show few

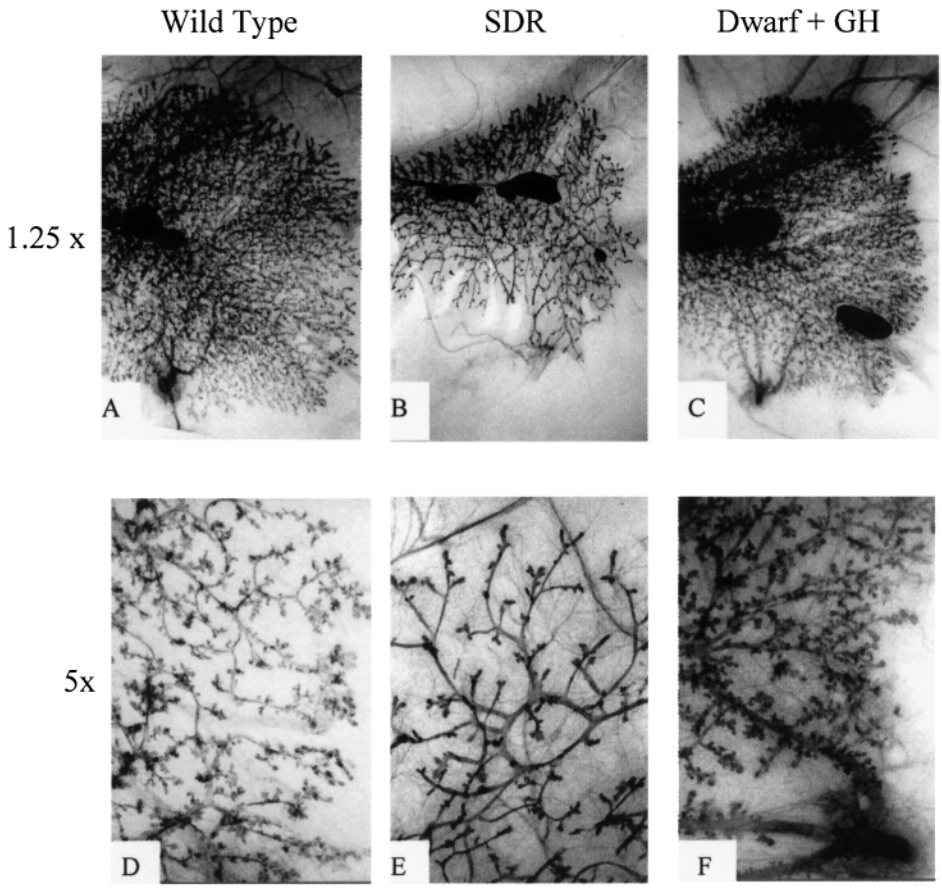

Fig. 3. Mammary gland whole mounts from 7-week-old rats treated with either GH or GH vehicle for 7 days. (**A** and **D**) Wild-type Sprague–Dawley rats treated with vehicle. (**B** and **E**) SDR treated with vehicle. The morphology of untreated SDR mammary glands was indistinguishable from vehicle treated glands. (**C** and **F**) SDR treated with GH. Original magnifications are indicated.

Table III. Summary of differences between SDR and wild-type Sprague–Dawley rats at 8 weeks of age (n = 10 rats/group)

Parameter	SDR	Wild-type	GH-treated dwarfs
Mammary gland area (cm^2)	1.98 ± 0.24	3.76 ± 0.35	3.88 ± 0.78
Mammary epithelial cell labeling index	7.5 ± 0.23	14.2 ± 1.39	15.3 ± 2.12
Mammary ductal branch points/mm^2	2.5 ± 0.07	2.6 ± 0.07	2.7 ± 0.06
Terminal end buds/mm^2	2.5 ± 0.15	2.6 ± 0.14	2.8 ± 0.17
Age at vaginal opening (days)	47.7 ± 0.30	36.3 ± 0.35	NA

Values are means ± SEM. NA, not assayed. Mammary gland area and epithelial cell proliferation in the SDR were significantly different (at 5% level) from either wild-type or GH treated SDRs; wild-type rats were not significantly different from GH treated SDRs (one-way ANOVA). Neither mammary ductal branching nor terminal end bud density was significantly different among the groups (one-way ANOVA, $P > 0.05$). Vaginal opening in the SDR was significantly delayed (Student's t-test; $P < 0.0001$).

alveolar buds (Figure 3B and E), indicating a role for GH in alveolar development in the virgin rat mammary gland.

To examine this possibility, we administered GH to SDR rats by continuous subcutaneous infusion. Body weights of SDRs treated with GH (n = 3) increased from 60 ± 2 to 92 ± 3 g (mean ± SEM) in 1 week, while SDRs treated with vehicle alone (n = 3) gained only 2 g on average. Upon stimulation with GH for 1 week, the mammary glands of SDRs respond with vigorous ductal growth and alveolar development (Figure 3C and F) yielding a morphology similar to that of the wild-type Sprague–Dawley. Proliferation as determined by BrdU labeling index paralleled the whole mount observations (Table III). As shown in Table III and Figure 3, proliferation in the SDR rats was about one-half that of the wild-type and increased with GH administration, demonstrating that GH stimulates proliferation in the mammary gland of SDR rats.

Discussion

We report that the SDR is markedly resistant to chemically induced mammary carcinogenesis and that in the young virgin SDR, development of alveolar buds appears to be stunted relative to that observed in the young virgin Sprague–Dawley rat. The SDR is a unique model of isolated GH deficiency (14) that arose from a colony of Sprague–Dawley rats (10,11); a strain that is highly susceptible to mammary carcinogenesis induced by a variety of chemical and physical agents (27). As the truncated transcript of the GH gene is expressed at only 6% the level observed in wild-type Sprague–Dawley pituitaries, and the GH protein is not detectable in serum, the SDR is, for all practical purposes, a 'natural knockout' rat for GH. A rat model of isolated GH deficiency such as the SDR is particularly valuable as rat mammary carcinogenesis is similar to human breast cancer in many important ways including tumor site of origin, histology and response to SERMS or endocrine ablation.

The resistance of the SDR to mammary cancer is consistent with published results of both epidemiologic and laboratory-

based studies suggesting that the GH/IGF axis plays a role in carcinogenesis. Epidemiologic studies suggest that increased serum titers of IGF-I are associated with elevated risk to the most common and deadly forms of cancer in man (9), including breast cancer (28,29). Using nude mouse xenograft models, Schally *et al.* (30,31) have demonstrated that down-regulators of the GH/IGF axis such as GH releasing hormone antagonists or somatostatin analogs can inhibit the growth of human cancer cells derived from lung, ovary or prostate. Furthermore, these substances were effective at inhibiting the growth of estrogen-independent human breast cancer cells (32). Pollak *et al.* (33) found that human breast cancer (MCF-7) cells grow significantly more slowly in mice homozygous for the *lit* mutation relative to wild-type mice. The *lit* mutation results in loss of function of the pituitary GH-releasing hormone receptor and secondary suppression of GH and IGF-I.

We found that alveolar development in the mammary glands of virgin wild-type Sprague–Dawley rats is substantially more advanced than observed in the SDR mammary glands. Administration of GH to the SDR induces alveolar development resulting in mammary morphology indistinguishable from that of the wild-type Sprague–Dawley rat. The GH/IGF axis regulates normal mammary gland growth and development in many species (34). Receptors for GH have been identified in mouse (35), rat (36), monkey (37) and human (38) mammary stroma. While the majority of receptors are found on stromal cells, epithelial cells also express GH receptor. GH can also stimulate mammary cancer cells directly. Mertani *et al.* (38) recently reported that GH receptor expression is up-regulated during breast cancer progression in women. The IGFs, their receptors and binding proteins are found during all stages of normal mammary gland development in all mammalian species examined to date (39). Kleinberg *et al.* have shown that rat GH, acting through its receptors on mammary stromal cells (26), induces IGF-I that can act in a paracrine fashion to stimulate parenchymal proliferation and differentiation (8).

The data reported here dramatically demonstrate that GH is required for chemically induced mammary carcinogenesis in the rat. We used MNU and DMBA as carcinogens for these studies as they cause rat mammary cancers that have been exhaustively characterized. In addition, these chemicals induce cancer by different mechanisms. MNU is a direct-acting agent. It spontaneously breaks down at physiologic pH to generate a carbonium ion capable of methylating DNA. DMBA, however, requires metabolic activation by the cellular p450 system resulting in an electrophilic intermediate capable of producing bulky DNA adducts. MNU and DMBA generate distinct genetic lesions as well. For example, Ha-*ras* mutations are observed in both MNU- and, to a lesser extent, DMBA-induced mammary cancers. However, G^{35} to A transition mutations are observed in MNU-induced cancers, whereas A^{182} or A^{183} to T transversion mutations are observed in DMBA-induced mammary cancers (40). The fact that the absence of GH prevents tumors induced by carcinogens that activate Ha-*ras* at different frequencies suggests that inhibition is Ha-*ras* independent.

The mechanism(s) by which GH deficiency blocks rat mammary carcinogenesis remains to be determined. Data from the literature cited above suggest that the GH/IGF axis may be required for all phases of carcinogenesis from initial transformation through to the maintenance and progression of advanced cancers. Our finding that there are very few tumors or preneoplastic lesions observed in the chemically exposed SDR suggests that GH or the GH/IGF axis may be required for early events in mammary carcinogenesis. The smaller size of the SDR mammary gland means that there may be fewer target cells for carcinogens to transform. However, the size of the SDR mammary glands are only about half the size of the those in wild-type rats, yet the difference in cancer multiplicity is ~20-fold (Figure 1). Furthermore, the mammary glands of 21-day-old wild-type Sprague–Dawley rats are smaller than 7-week-old SDRs, yet are highly susceptible to neoplastic transformation (41). Nevertheless, as suggested by the clear morphologic differences observed between the SDR and wild-type Sprague–Dawley mammary glands, GH may be required for the development and/or maintenance of mammary epithelial cells that are targets for oncogenic agents.

Another possible means by which the absence of GH may block carcinogenesis is by altering the response of mammary epithelial cells to carcinogenic insult. Survival factors such as IGF-I initiate a signaling cascade leading to the activation of serine kinases that modulate the activity of members of the Bcl-2 family, which regulates the apoptotic machinery in most cells (42) including mammary epithelial cells (43). Therefore, carcinogen-exposed mammary cells in wild-type animals may have a relative survival advantage not enjoyed by similarly treated cells in GH deficient rats. The fact that the few cancers that are observed in the SDR are very small (only a few mm^3) suggests that GH may also be required for later events in tumor progression. Based on these findings, it is reasonable to speculate that novel therapeutics targeting the GH/IGF pathway may be effective in the prevention and/or treatment of breast cancer.

Acknowledgements

The authors thank Gwynne M.Kizer, Eulenia R.Lim and Daniel D.Lantvit for their expert technical assistance. The authors also wish to thank Drs Ron G.Rosenfeld and Sharron E.Gargosky for generously providing the Spontaneous Dwarf rats. This research was supported by Public Health Service Grants R29 CA71895 and R01 DK41430 from the National Institutes of Health, DHHS, the Department of Veterans Affairs Merit Review Program and by an award from the Hans Vahlteich Endowment, College of Pharmacy, University of Illinois at Chicago.

References

1. Greenlee,R.T., Hill-Harmon,M.B., Murray,T. and Thun,M. (2001) Cancer statistics, 2001. *CA Cancer J. Clin.*, **51**, 15–36.
2. Osborne,C.K., Zhao,H. and Fuqua,S.A. (2000) Selective estrogen receptor modulators: structure, function and clinical use. *J. Clin. Oncol.*, **18**, 3172–3186.
3. Pegram,M. and Slamon,D. (2000) Biological rationale for HER2/neu (c-erbB2) as a target for monoclonal antibody therapy. *Semin. Oncol.*, **27**, 13–19.
4. Osborne,C.K. (1998) Steroid hormone receptors in breast cancer management. *Breast Cancer Res. Treat.*, **51**, 227–238.
5. Slamon,D.J., Clark,G.M., Wong,S.G., Levin,W.J., Ullrich,A. and McGuire,W.L. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, **235**, 177–182.
6. Frei,E. and Antman,K.H. (2000) Principles of dose, schedule and combination chemotherapy. In Bast,R.C., Kufe,D.W., Pollock,R.E., Morton,D.L., Weichselbaum,R.R., Holland,J.F. and Frei,E. (eds) *Cancer Medicine*, 5th Edn. BC Decker, London, pp. 556–568.
7. Thordarson,G., Jin,E., Guzman,R.C., Swanson,S.M., Nandi,S. and Talamantes,F. (1995) Refractoriness to mammary tumorigenesis in parous rats: is it caused by persistent changes in the hormonal environment or permanent biochemical alterations in the mammary epithelia? *Carcinogenesis*, **16**, 2847–2853.
8. Walden,P.D., Ruan,W., Feldman,M. and Kleinberg,D.L. (1998) Evidence that the mammary fat pad mediates the action of growth hormone in mammary gland development. *Endocrinology*, **139**, 659–662.

9. Pollak,M. (2000) Insulin-like growth factor physiology and neoplasia. *Growth Horm. IGF Res.*, **10** (suppl. A), S6–7.
10. Okuma,S. (1980) Spontaneous dwarf rat. *Exp. Anim.*, **29**, 301–305.
11. Okuma,S. (1984) Study of growth hormone in spontaneous dwarf rat. *Nippon Naibunpi Gakkai Zasshi*, **60**, 1005–1014.
12. Takeuchi,T., Suzuki,H., Sakurai,S., Nogami,H., Okuma,S. and Ishikawa,H. (1990) Molecular mechanism of growth hormone (GH) deficiency in the spontaneous dwarf rat: detection of abnormal splicing of GH messenger ribonucleic acid by the polymerase chain reaction. *Endocrinology*, **126**, 31–38.
13. Nogami,H., Suzuki,K., Matsui,K., Ookuma,S. and Ishikawa,H. (1989) Electron-microscopic study on the anterior pituitary gland of spontaneous dwarf rats. *Cell Tissue Res.*, **258**, 477–482.
14. Nogami,H., Takeuchi,T., Suzuki,K., Okuma,S. and Ishikawa,H. (1989) Studies on prolactin and growth hormone gene expression in the pituitary gland of spontaneous dwarf rats. *Endocrinology*, **125**, 964–970.
15. Gargosky,S.E., Nanto-Salonen,K., Tapanainen,P. and Rosenfeld,R.G. (1993) Pregnancy in growth hormone-deficient rats: assessment of insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs) and IGFBP protease activity. *J. Endocrinol.*, **136**, 479–489.
16. Thompson,H.H. (2000) Methods for the induction of mammary carcinogenesis in the rat usng either 7,12-dimethylbenz[a]anthracene or 1-methyl-1-nitrosourea. In Ip,M.M. and Asch,B.B. (eds) *Methods in Mammary Gland Biology and Breast Cancer Research*. Kluwer, New York, pp. 19–29.
17. Institute of Laboratory Animal Resources (US) (1996) *Guide for the Care and Use of Laboratory Animals*, 7th Edn. National Academy Press, Washington, DC.
18. Russo,J. and Russo,I.H. (2000) Atlas and histologic classification of tumors of the rat mammary gland. *J. Mammary Gland Biol. Neoplasia*, **5**, 187–200.
19. Steel,G.G. (1977) Growth rate of tumours. In *Growth Kinetics of Tumours*. Clarendon Press, Oxford, pp. 5–55.
20. Armitage,P. (1966) The chi-square test for heterogeneity of proportion after adjustment for stratification. *J. R. Stat. Soc. [Ser. B]*, **28**, 150–163.
21. Nogami,H., Watanabe,T. and Takeuchi,T. (1992) Effect of growth hormone (GH) on the promotion of body weight gain in the spontaneous dwarf rat: a novel experimental model for isolated GH deficiency. *Horm. Metab. Res.*, **24**, 300–301.
22. Kamegai,J., Unterman,T.G., Frohman,L.A. and Kineman,R.D. (1998) Hypothalamic/pituitary-axis of the spontaneous dwarf rat: autofeedback regulation of growth hormone (GH) includes suppression of GH releasing-hormone receptor messenger ribonucleic acid. *Endocrinology*, **139**, 3554–3560.
23. Rasmussen,S.B., Young,L.J.T. and Smith,G.H. (2000) Preparing mammary gland whole mounts from mice. In Ip,M.M. and Asch,B.B. (eds) *Methods in Mammary Gland Biology and Breast Cancer Research*. Kluwer, New York, pp. 75–85.
24. Russo,J., Russo,I.H., van Zwieten,M.J., Rogers,A.E. and Gusterson,B. (1989) Classification of neoplastic and nonneoplastic lesions of the rat mammary gland. In *Monographs on Pathology of Laboratory Animals. Integument and Mammary Glands.* Springer-Verlag, Berlin, pp. 275–304.
25. McGinley,J.N., Knott,K.K. and Thompson,H.J. (2000) Effect of fixation and epitope retrieval on BrdU indices in mammary carcinomas. *J. Histochem. Cytochem.*, **48**, 355–362.
26. Feldman,M., Ruan,W., Cunningham,B.C., Wells,J.A. and Kleinberg,D.L. (1993) Evidence that the growth hormone receptor mediates differentiation and development of the mammary gland. *Endocrinology*, **133**, 1602–1608.
27. Russo,J. and Russo I.H. (1996) Experimentally induced mammary tumors in rats. *Breast Cancer Res. Treat.*, **39**, 7–20.
28. Hankinson,S.E., Willett,W.C., Colditz,G.A., Hunter,D.J., Michaud,D.S., Deroo,B., Rosner,B., Speizer,F.E. and Pollak,M. (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet*, **351**, 1393–1396.
29. Chan,J.M., Stampfer,M.J., Giovannucci,E., Gann,P.H., Ma,J., Wilkinson,P., Hennekens,C.H. and Pollak,M. (1998) Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science*, **279**, 563–566.
30. O'Byrne,K.J., Schally,A.V., Thomas,A., Carney,D.N. and Steward,W.P. (2001) Somatostatin, its receptors and analogs, in lung cancer. *Chemotherapy*, **47** (suppl. 2), 78–108.
31. Schally,A.V., Comaru-Schally,A.M., Plonowski,A., Nagy,A., Halmos,G. and Rekasi,Z. (2000) Peptide analogs in the therapy of prostate cancer. *Prostate*, **45**, 158–166.
32. Kahan,Z., Varga,J.L., Schally,A.V., Rekasi,Z., Armatis,P., Chatzistamou,L., Czompoly,T. and Halmos,G. (2000) Antagonists of growth hormone-releasing hormone arrest the growth of MDA-MB-468 estrogen-independent human breast cancers in nude mice. *Breast Cancer Res. Treat.*, **60**, 71–79.
33. Yang,X.F., Beamer,W.G., Huynh,H. and Pollak,M. (1996) Reduced growth of human breast cancer xenografts in hosts homozygous for the lit mutation. *Cancer Res.*, **56**, 1509–1511.
34. Plaut,K., Ikeda,M. and Vonderhaar,B.K. (1993) Role of growth hormone and insulin-like growth factor-I in mammary development. *Endocrinology*, **133**, 1843–1848.
35. Ilkbahar,Y.N., Wu,K., Thordarson,G. and Talamantes,F. (1995) Expression and distribution of messenger ribonucleic acids for growth hormone (GH) receptor and GH-binding protein in mice during pregnancy. *Endocrinology*, **136**, 386–392.
36. Lincoln,D.T., Water,M.J., Breipohl,W., Sinowatz,F. and Lobie,P.E. (1990) Growth hormone receptors expression in the proliferating rat mammary gland. *Acta Histochem. Suppl.*, **40**, 47–49.
37. Ng,S.T., Zhou,J., Adesanya,O.O., Wang,J., LeRoith,D. and Bondy,C.A. (1997) Growth hormone treatment induces mammary gland hyperplasia in aging primates. *Nature Med.*, **3**, 1141–1144.
38. Mertani,H.C., Garcia-Caballero,T., Lambert,A., Gerard,F., Palayer,C., Boutin,J.M., Vonderhaar,B.K., Waters,M.J., Lobie,P.E. and Morel,G. (1998) Cellular expression of growth hormone and prolactin receptors in human breast disorders. *Int. J. Cancer*, **79**, 202–211.
39. Wood,T.L. and Yee,D. (2000) Introduction: IGFs and IGFBPs in the normal mammary gland and in breast cancer. *J. Mammary Gland Biol. Neoplasia*, **5**, 1–5.
40. Sukumar,S. (1989) ras oncogenes in chemical carcinogenesis. *Curr. Top. Microbiol. Immunol.*, **148**, 93–114.
41. Thompson,H.J., McGinley,J.N., Rothhammer,K. and Singh,M. (1995) Rapid induction of mammary intraductal proliferations, ductal carcinoma *in situ* and carcinomas by the injection of sexually immature female rats with 1-methyl-1-nitrosourea. *Carcinogenesis*, **16**, 2407–2411.
42. O'Connor,R., Fennelly,C. and Krause,D. (2000) Regulation of survival signals from the insulin-like growth factor-I receptor. *Biochem. Soc. Trans.*, **28**, 47–51.
43. Neuenschwander,S., Schwartz,A., Wood,T.L., Roberts,C.T.Jr, Henninghausen,L. and LeRoith,D. (1996) Involution of the lactating mammary gland is inhibited by the IGF system in a transgenic mouse model. *J. Clin. Invest.*, **97**, 2225–2232.

Received December 22, 2001; revised March 8, 2002; accepted March 12, 2002