Reduced Growth of Human Breast Cancer Xenografts in Hosts Homozygous for the *lit* Mutation[1]

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Abstract

Insulin-like growth factor I (IGF-I) is a potent breast cancer mitogen. Growth hormone (GH) up-regulates hepatic *IGF-I* gene expression and circulating IGF-I level. Tissue IGF bioactivity is influenced not only by circulating IGF-I and IGF-II levels but also by autocrine and paracrine production of these growth factors and by IGF binding proteins. There is considerable person-to-person variability in GH-IGF-I physiology. Both laboratory and epidemiological data are consistent with the hypothesis that the host GH-IGF-I axis influences breast cancer behavior, but such an effect has not been directly demonstrated. To determine whether breast cancer growth in an *in vivo* model is influenced by the host GH-IGF-I axis, we compared the growth of human MCF-7 breast cancer cells in control mice to that in mice homozygous for *lit*, a missense mutation resulting in loss of function of the pituitary GH-releasing hormone receptor and secondary suppression of GH and IGF-I. Breast cancer growth was significantly reduced in *lit/lit* animals compared to control hosts [tumor size (mean ± SD) on day 39, 444 ± 82 *versus* 845 ± 444 mm^3, respectively; $P < 0.001$, Mann-Whitney U test]. These data demonstrate that in our model, host GH-IGF-I axis physiology plays a role in determining breast cancer behavior. The results *a*) suggest that patient-to-patient variability in GH-IGF-I physiology may contribute to the large variability between patients regarding breast cancer behavior, and *b*) motivate clinical trials of novel hormonal treatment strategies that target the GH-IGF-I axis.

Introduction

Both host factors and tumor factors contribute to the substantial variability between individuals with respect to the clinical course of breast cancer. Whereas many genetic derangements in breast cancer cells that adversely affect prognosis have been described, few host factors that influence breast cancer behavior have been identified. There is considerable variability between individuals with respect to function of the GH[3]-IGF-I axis (1). It has been proposed on the basis of experimental and epidemiological data that this heterogeneity may account for a portion of the variability between individuals with respect to breast cancer prognosis, and also may be related to breast cancer risk (2, 3). As variability in the GH-IGF-I axis contributes to differences between individuals with respect to body structure (1), data demonstrating a positive correlation between height and breast cancer risk (4–9, reviewed in Ref. 10) also support the hypothesis that IGF-I physiology, particularly during or subsequent to the pubertal period of breast and skeletal development, is related to risk. Clinical evidence for an association between large body size and aggressive breast cancer behavior is weaker (4, 5, 7), but this is not unexpected given the anticipated influence of potentially confounding variables such as treatment.

GH is a weak mitogen for human breast cancer cells relative to other peptide growth factors (11). On the other hand, significant stimulation of *in vitro* breast cancer cell proliferation by nanomolar concentrations of IGF-I (12, 13) and reduction of *in vivo* MCF-7 cell breast tumor growth by a blocking antibody directed against the type I IGF receptor (14) provide strong evidence that IGF-I is a potent mitogen for breast cancer cells. However, these data are not sufficient to demonstrate a role for the host GH-IGF-I axis in determining the behavior of breast cancer, in part because IGF-I or IGF-II from autocrine or paracrine sources in the microenvironment of neoplastic cells (15) may render systemic IGF-I levels irrelevant. Taken together, epidemiological and experimental evidence to date is compatible with but does not establish an influence of the host GH-IGF-I axis on breast cancer behavior.

To determine whether the host GH/IGF-I axis influences the behavior of human breast neoplasms, we developed a stock of mice homozygous for the *scid* mutation (16) and segregating for the little (*lit*) mutation (17). Recent studies have characterized the *lit* mutation as an A→G transition in the GH-releasing hormone receptor gene resulting in substitution of Gly for Asp[60]. This results in loss of signal transduction function (18–20), a severe but highly specific deficit in pituitary GH synthesis and output, and a secondary reduction in *IGF-I* gene expression and serum level. Mice of this stock are either *scid/scid lit/lit* (GH-IGF-I deficient) or *scid/scid lit/+* (GH/IGF-I replete) and are able to act as hosts for human MCF-7 breast cancer xenografts. This mutation has recently been detected in humans as well as in mice (21).

Materials and Methods

Mice. A stock of C57BL/6 mice homozygous for the *scid* mutation (16) and segregating for the little (*lit*) mutation (17) were raised in a research colony (W. B.) at The Jackson Laboratory on acidified water and NIH 31 modified (6% fat) diet (Agway). Environmental conditions included lighting cycles of 14 h duration and ambient temperature range of 23 ± 3°C. In all studies, we used 3–4-month-old virgin females segregating for the *lit* mutation. Studies of MCF-7 xenograft growth were carried out with mice that were homozygous for the *scid* mutation and either homozygous for *lit* (*scid/scid lit/lit*) or heterozygous for *lit* (*scid/scid lit/+*). Sera for studies of MCF-7 cell growth *in vitro* were obtained from by cardiac puncture at necropsy.

***In Vivo* Tumor Growth.** 5×10^6 MCF-7 cells were injected s.c. in the mammary fat pad of 12-week-old *scid/scid lit/lit* female mice ($n = 18$) and *scid/scid lit/+* controls ($n = 17$). All mice received standard estradiol supplementation for the MCF7 murine xenograft model. This was accomplished by means of a 6-mm (length) by 0.1-mm (internal diameter) s.c.-implanted estradiol-packed silicone plastic tube. Tumors were measured with calipers, and volumes were calculated in mm^3 as (width-1) × (length-1)^2. This work was approved by the McGill animal care committee, with tumor size limited to 1 cm^3.

***In Vitro* Cell Proliferation.** MCF-7 cells were obtained from the American Type Tissue Culture Collection (Rockville, Maryland). Stock cultures were grown in Alpha medium (Life Technologies, Inc., Grand Island, NY) supplemented with 10% FCS (Life Technologies, Inc.) and 5 μg/ml bovine insulin (Sigma Chemical Co., St. Louis, MO). For proliferation studies, cells were plated at 2.5 × 104 cells/2-cm well in 24-well plates in Alpha medium supplemented with 2.5% FCS for 48 h. Cells were then washed with serum-free medium and then allowed to proliferate in the presence of specified concentrations of human recombinant IGF-I (a kind gift from Celtrix Pharmaceuticals) or serum obtained from donor mice by cardiac puncture at time of necropsy. Cells were then trypsinized, suspended in PBS, and counted with a hemocytometer.

IGF-I RIA. Sera from control and *lit/lit* animals were pooled after sacrifice. IGF-I level of each pool was determined by RIA (2) after separation of IGF binding proteins by low-pressure acid chromatography (22).

Results and Discussion

As expected, body weights, nose-tail length, and serum IGF-I concentration were reduced in young adult *scid/scid lit/lit* mice compared to *scid/scid lit/+* littermate controls (Table 1). Growth of MCF-7 xenografts was significantly reduced in the *lit/lit* hosts relative to controls ($P < 0.05$ for each day between 26 and 40; global $P < 0.001$, Mann-Whitney U test; Table 1; Fig. 1). All mice received equal estradiol supplementation, as is standard for the MCF-7 xenograft model. Consistent with reduced xenograft growth was our finding that serum from *lit/lit* mice was less mitogenic for MCF-7 cells than serum from littermate *lit/+* controls (Fig. 2). The difference in mitogenicity of control and *lit/lit* serum could be abolished by the addition of IGF-I (≥ 5 ng/ml) to the *lit/lit* serum (Fig. 2).

It has recently been recognized that tamoxifen, the most frequently used drug in breast cancer treatment, suppresses pituitary GH output and IGF-I levels, but to a lesser degree than does the *lit* mutation (2, 23, 24). Our results are consistent with the possibility that this effect of tamoxifen contributes to its antineoplastic action.

Appreciation of the influence of host ovarian function on breast cancer behavior led to the development of many endocrine therapies currently used in breast cancer management. In this context, the data reported here suggest examination of strategies to therapeutically exploit the influence of the host GH-IGF-I axis. There is evidence that it is possible to pharmacologically mimic the suppression of the GH-IGF-I axis associated with the *lit* mutation by the use of GH-releasing hormone antagonists (25), somatostatin analogues (26, 27), or competitive antagonists to receptors for GH or IGF-I (28, 29). Such treatments would represent "medical hypophysectomy" selective for the GH-IGF-I axis. Older clinical reports (30) documented a significant palliative effect of hypophysectomy in breast cancer, even after prior oophorectomy and adrenalectomy. Our results suggest that such responses may have been a consequence of ablation of the GH-IGF-I axis. Although gene knockout experiments suggest that the total absence of IGF-I is often lethal (31), the less-severe IGF-I deficiency resulting from the *lit* mutation is well tolerated by mice, in keeping with clinical evidence that sequellae of GH deficiency are mild relative to the toxicity of most antineoplastic therapies.

The impaired growth of breast cancer in hosts homozygous for the *lit* mutation demonstrates an influence of the host GH-IGF-I axis on breast cancer behavior. Many genes apart from the GH-releasing

Table 1 *Characteristics of lit/lit and control mice and growth of MCF-7 xenografts*

Length (nose to end of tail) was determined by caliper measurements and weight by electronic balance before xenograft implantation. Serum IGF-I was measured by RIA of five aliquots of serum pooled from animals of each group. Significance of differences between groups was determined by Mann-Whitney U test.

	Control (n = 17)	*lit/lit* (n = 18)
Length, cm	17.8 ± 0.2	14.6 ± 0.20[a]
Weight, g	24.8 ± 1.6	17.4 ± 0.9[a]
Serum IGF-I, ng/ml	903.7 ± 20.9	49.3 ± 5.3[a]
Mean tumor size on day 39, mm^3	845 ± 98	444 ± 82[a]
Mean time for last doubling of tumor volume, days	8	13[a]
% tumors <500 mm^3 on day 39	18	56
% tumors >1000 mm^3 on day 39	35	5

[a] $P < 0.05$.

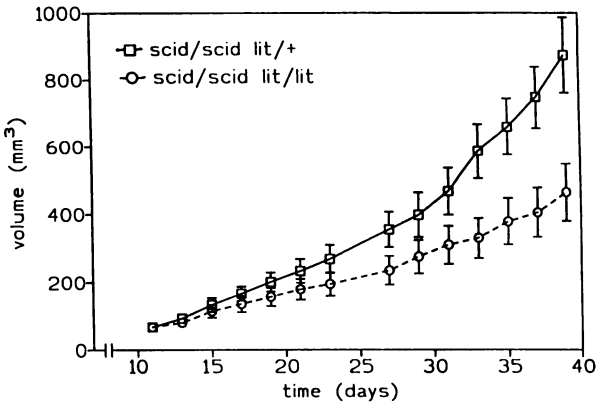

Fig. 1. Growth of human MCF-7 breast cancer xenografts in *scid/scid lit/+* control and *scid/scid lit/lit* mice. Cells (5 × 10⁶) were injected into the mammary fat pads of mice on day 0, and tumor growth was measured with calipers as described in "Materials and Methods." *Data points*, mean; *bars*, SD.

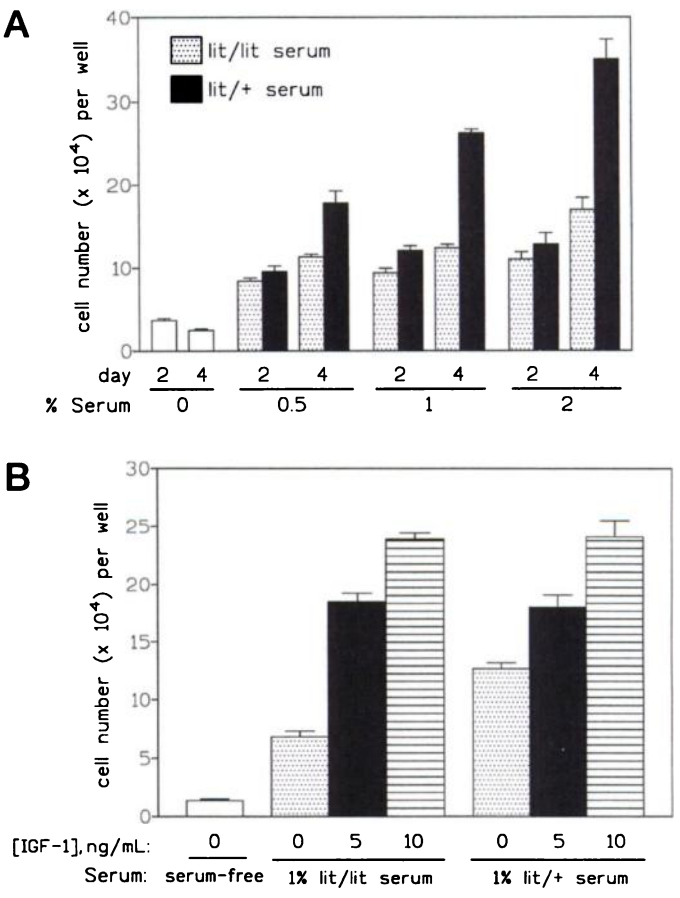

Fig. 2. *In vitro* growth of MCF-7 cells in the presence of sera from GH/IGF-I deficient *lit/lit* or normal *lit/+* mice. *A*, 2.5 × 10⁴ cells were allowed to proliferate for either 2 or 4 days in the presence of the indicated concentrations of serum from *lit/+* or *lit/lit* mice before determination of cell number. *B*, 2.5 × 10⁴ cells were allowed to proliferate for 2 days in the presence of the indicated concentrations of serum from control or *lit/lit* mice and the indicated concentration of IGF-I before determination of cell number. *Columns*, mean; *bars*, SD.

hormone receptor gene to which the *lit* mutation has been mapped are involved in regulation of IGF physiology. Although loss-of-function mutations such as *lit* represent an extreme example, polymorphic variation of various genes involved in regulating host IGF physiology (including type I and type II IGF receptors and the IGF binding proteins) may influence behavior of breast cancer, either by direct effects on neoplastic cells or by an influence on processes such as angiogenesis that involve host-tumor interactions. The *lit* mutation represents an instance of subnormal activity of the GH-IGF-I axis associated with reduced somatic growth, decreased fecundity, and less aggressive breast cancer behavior. Selective pressure in human populations, in contrast, may have favored alleles that are associated with enhanced reproductive fitness and relatively high IGF-I bioactivity but predispose to higher incidence and/or more aggressive behavior of breast cancer. A report (32) of higher IGF-I serum levels in women with breast cancer than in matched controls is consistent with this possibility.

Finally, the results reported here may have relevance to the influence of dietary restriction on breast cancer behavior, as reduced caloric intake in rodent mammary tumor models is associated with both suppression of IGF-I levels and suppression of neoplastic growth (33).

Acknowledgments

We thank Bonnie Deroo for assistance in this work.

References

1. Juul, A., Bang, P., Hertel, N., *et al.* Serum insulin-like growth factor I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J. Clin. Endocrinol. Metab., *78:* 744–752, 1995.
2. Pollak, M., Costantino, J., Polychronakos, C., Blauer, S., Guyda, H., Redmond, C., Fisher, B., and Margolese, R. Effect of tamoxifen on serum insulin-like growth factor I levels in stage I breast cancer patients. J. Natl. Cancer Inst., *82:* 1693–1697, 1990.
3. Stoll, B. Breast cancer risk in Japanese women with special reference to the growth hormone-insulin-like growth factor axis. Jpn. J. Clin. Oncol., *22:* 1–5, 1992.
4. deWaard, F., Cornelis, J., Aoki, K., and Yoshida, M. Breast cancer incidence according to weight and height in two cities of the Netherlands and in Aichi prefecture. Jpn. Cancer, *40:* 1269–1275, 1995.
5. Vatten, L., Kvikstad, A., and Nymoen, E. Incidence and mortality of breast cancer related to body height and living conditions during childhood and adolescence. Eur. J. Cancer, *28:* 128–131, 1992.
6. Murata, M., Kuno, K., and Sakamoto, G. Epidemiology of family predisposition for breast cancer in Japan. J. Natl. Cancer Inst., *69:* 1229–1234, 1982.
7. Tretli, S. Height and weight in relation to breast cancer morbidity and mortality. A prospective study of 570,000 women in Norway. Int. J. Cancer, *44:* 23–30, 1989.
8. Vatten, L. J., and Kvinnsland, S. Body height and risk of breast cancer. A prospective study of 23,831 Norwegian women. Br. J. Cancer, *61:* 881–885, 1990.
9. Mondina, R., Borsellino, G., Poma, S., Baroni, M., Di Nubila, B., and Sacchi, P. Breast carcinoma and skeletal formation. Eur. J. Cancer, *28A:* 1068–1070, 1992.
10. Hunter, D., and Willett, W. Diet, body size, and breast cancer. Epidemiol. Rev., *15:* 110–132, 1993.
11. Shiu, R., and Paterson, J. Alteration of cell shape, adhesion, and lipid accumulation in human breast cancer cells by human prolactin and growth hormone. Cancer Res., *44:* 1178–1186, 1984.
12. Pollak, M., Polychronakos, C., Yousefi, S., and Richard, M. Characterization of insulin-like growth factor (IGF-I) receptors of human breast cancer cells. Biochem. Biophys. Res. Commun., *154:* 326–331, 1988.
13. Arteaga, C. L., and Osborne, C. K. Growth inhibition of human breast cancer cells *in vitro* with an antibody against the type I somatomedin receptor. Cancer Res., *49:* 6237–6241, 1989.
14. Arteaga, C. L., Kitten, L. J., Coronado, E. B., Jacobs, S., Kull, F. C., Allred, D. C., and Osborne, C. K. Blockade of the type I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. J. Clin. Invest., *84:* 1418–1423, 1989.
15. Yee, D., Paik, S., Lebovic, G. S., Marcus, R. R., Favoni, R. E., Cullen, K. J., Lippman, M. E., and Rosen, N. Analysis of insulin-like growth factor I gene expression in malignancy: evidence for a paracrine role in human breast cancer. Mol. Endocrinol., *3:* 517–519, 1989.
16. Schuler, W., and Bosma, M. J. Nature of the scid defect: a defective VDJ recombinase system. Curr. Top. Microbiol. Immunol., *152:* 55–62, 1989.
17. Eicher, E., and Beamer, W. Inherited ateliotic dwarfism in mice. Characteristics of the mutation, little, on chromosome 6. Journal of Heredity, *67:* 87–91, 1976.
18. Jansson, J. O., Downs, T. R., Beamer, W. G., and Frohman, L. A. Receptor-associated resistance to growth hormone-releasing factor in dwarf "little" mice. Science (Washington DC), *232:* 511–512, 1986.
19. Godfrey, P., Rahal, J. O., Beamer, W. G., Copeland, N. G., Jenkins, N. A., and Mayo, K. E. GHRH receptor of little mice contains a missense mutation in the extracellular domain that disrupts receptor function. Nat. Genet., *4:* 227–232, 1993.
20. Lin, S. C., Lin, C. R., Gukovsky, I., Lusis, A. J., Sawchenko, P. E., and Rosenfeld, M. G. Molecular basis of the little mouse phenotype and implications for cell type-specific growth. Nature (Lond.), *364:* 208–213, 1993.
21. Wajnrajch, M. P., Gertner, J. M., Harbison, M. D., Chua, S. C., and Leibel, R. L. Nonsense mutation in the human growth hormone releasing hormone receptor causes growth failure analogous to the little (lit) mouse. Nat. Genet., *12:* 88–90, 1996.
22. Bang, P., Baxter, R. C., Blum, W. F., Breier, B. H., Clemmons, D. R., Hall, K., Hintz, R. L., Holly, J. M., Rosenfeld, R. G., and Zapf, J. Valid measurements of total IGF concentrations in biological fluids. Recommendations from the 3rd International Symposium on Insulin-like Growth Factors. Endocrinology, *136:* 816–817, 1995.
23. Tannenbaum, G. S., Gurd, W., Lapointe, M., and Pollak, M. Tamoxifen attenuates pulsatile growth hormone secretion: mediation in part by somatostatin. Endocrinology, *130:* 3395–3401, 1992.
24. Huynh, H. T., Tetenes, E., Wallace, L., and Pollak, M. *In vivo* inhibition of insulin-like growth factor-I gene expression by tamoxifen. Cancer Res., *53:* 1727–1730, 1993.
25. Zarandi, M., Horvath, J. E., Halmos, G., Pinski, J., Nagy, A., Groot, K., Rekasi, Z., and Schally, A. V. Synthesis and biological activities of highly potent antagonists of growth hormone-releasing hormone. Proc. Natl. Acad. Sci. USA, *91:* 12298–12302, 1994.
26. Huynh, H. T., and Pollak, M. Enhancement of tamoxifen-induced suppression of insulin-like growth factor I gene expression and serum level by a somatostatin analogue. Biochem. Biophys. Res. Commun., *203:* 253–259, 1994.
27. Weckbecker, G., Tolcsvai, L., Stolz, B., Pollak, M., and Bruns, C. Somatostatin analogue octreotide enhances the antineoplastic effects of tamoxifen and ovariectomy on 7,12-dimethylbenz(*a*)anthracene-induced rat mammary carcinomas. Cancer Res., *54:* 6334–6337, 1994.
28. Chen, W. Y., Chen, N. Y., Yum, J., Washer, T. E., and Kopchick, J. J. *In vitro* and *in vivo* studies of antagonistic effects of human growth hormone analogues. J. Biol. Chem., *269:* 15892–15897, 1994.
29. Pietrzkowski, Z., Wernicke, D., Porcu, P., Jameson, B. A., and Basensa, R. Inhibition of cellular proliferation by peptide analogs of IGF-I. Cancer Res., *52:* 6447–6451, 1992.
30. Ray, B. S., and Pearson, O. H. Hypophysectomy in treatment of disseminated breast cancer. Surg. Clin. North Am., *12:* 419–433, 1962.
31. Liu, J. P., Baker, J., Perkins, A. S., Robertson, E. J., and Efstratiadis, A. Mice carrying null mutations of the genes encoding insulin-like growth factor (IGF-1) and type 1 IGF receptor (IGF1r). Cell, *75:* 59–72, 1993.
32. Peyrat, J. P., Bonneterre, J., Hecquet, B., Vennin, P., Louchez, M. M., Fournier, C., Lefebvre, J., and Demaille, A. Plasma insulin-like growth factor-1 (IGF-1) concentration in human breast cancer. Eur. J. Cancer, *29A:* 492–497, 1993.
33. Ruggeri, B., Klurfeld, D., Kritchevsky, D., and Furlanetto, R. Caloric restriction and DMBA-induced mammary tumor growth in rats: alternations in circulating insulin, insulin-like growth factors I and II, and epidermal growth factor. Cancer Res., *49:* 4130–4134, 1989.