Tumor Expression of Human Growth Hormone and Human Prolactin Predict a Worse Survival Outcome in Patients with Mammary or Endometrial Carcinoma

Zheng-Sheng Wu,* Kun Yang,* Yu Wan, Peng-Xu Qian, Jo K. Perry, Jean Chiesa, Hichem C. Mertani, Tao Zhu,* and Peter E. Lobie*

Hefei National Laboratory for Physical Sciences at Microscale (Z.-S.W., P.-X.Q., T.Z.), University of Science and Technology of China, Hefei, Anhui 230027, People's Republic of China; Department of Pathology (Z.-S.W.), Anhui Medical University, Hefei, Anhui 230032, People's Republic of China; Department of Pathology (Z.-S.W.), Shanghai Medical College, Fundan University, Shanghai 200032, People's Republic of China; Fourth Military Medical University (K.Y.), Xi'an 710032, People's Republic of China; Department of Physiology (Y.W.), School of Basic Medicine, Wuhan University, Wuhan 430072, People's Republic of China; Liggins Institute (J.K.P.), University of Auckland, 1142 Auckland, New Zealand; Centre Hospitalier Universitaire de Nîmes (J.C.), Service de Cytologie Clinique et Cytogénétique Hôpital Gaston Doumergue, 30029 Nîmes, France; Centre de Recherche en Cancérologie de Lyon, Unité Mixte de Recherche INSERM U 1052–CNRS 5286 (H.C.M.), Université de Lyon, Université Claude Bernard, 69366 Lyon 1, France; and Cancer Science Institute of Singapore and Department of Pharmacology (P.E.L.), National University of Singapore, Singapore117456

Context: Evidence suggests that human GH (hGH) and human prolactin (hPRL) possess an autocrine or paracrine oncogenic role in mammary and endometrial carcinoma. However, especially for hGH, the prognostic relevance of tumor expression of these hormones is not well defined.

Objective: We investigated the potential association of tumor mRNA and protein expression of hGH and hPRL with the clinicopathological features of mammary and endometrial carcinoma. The prognostic relevance of the individual or combined expression of hGH and hPRL in mammary and endometrial carcinoma was also determined.

Design: The expression of hGH and hPRL was analyzed in histopathological samples of mammary and endometrial carcinoma, and the respective normal tissues, by *in situ* hybridization and immunohistochemistry. Kaplan-Meier and Cox regression analysis was performed to examine the association of tumor hGH and hPRL expression with relapse-free survival and overall survival of patients.

Results: hGH expression was significantly associated with lymph node metastasis, tumor stage, human epidermal growth factor receptor-2 status, and proliferative index in mammary carcinoma and with International Federation of Gynecology and Obstetrics grade, myometrial invasion, and ovarian metastases in endometrial carcinoma. hPRL expression was associated with lymph node metastasis, tumor grade, and tumor stage in mammary carcinoma and with International Federation of Gynecology and Obstetrics stage and myometrial invasion in endometrial carcinoma. Both hGH and hPRL expression, individually and combined, are associated with worse relapse-free survival and overall survival in patients with mammary or endometrial carcinoma.

Conclusion: Tumor expression of both hGH or hPRL in mammary or endometrial carcinoma is associated with a large and significant difference in survival outcome for patients with these tumors. **(*J Clin Endocrinol Metab* 96: E1619–E1629, 2011)**

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* Z.-S.W., K.Y., T.Z., and P.E.L. contributed equally to this work.

Abbreviations: ER, Estrogen receptor; FIGO, International Federation of Gynecology and Obstetrics; hCS, human chorionic somatomammotrophin; HER-2, human epidermal growth factor receptor 2; hGH, human GH; hPL, human placental lactogen; hPRL, human prolactin; IHC, immunohistochemistry; ISH, *in situ* hybridization; OS, overall survival; pAb, polyclonal antibody; PR, progesterone receptor; RFS, relapse-free survival; TMA, tissue microarray.

A number of recent reviews (1–5) have summarized genetic, epidemiological, animal, and cell biology derived evidence implicating human GH (hGH) and human prolactin (hPRL) in development and progression of tumors of the female reproductive system among others. As an example, in a recent large genome-wide association study (6), the GH signaling pathway was identified as the pathway third most significantly correlated with susceptibility to develop mammary carcinoma.

In addition to the classical endocrine roles of pituitary derived hGH and hPRL, it is now widely accepted that these hormones also function in an autocrine or paracrine manner, as a local growth factor, in a number of tissues, including the mammary gland and endometrium (7). Pathological roles of autocrine/paracrine hGH and hPRL have been postulated in both mammary and endometrial carcinoma (1–5, 7–9). Indeed, increased expression of hGH in immortalized but otherwise normal human mammary epithelial cells has been reported to be sufficient to stimulate oncogenic transformation of these cells with consequent tumor formation in a xenograft model (10). Furthermore, autocrine expression of hGH stimulates mammary and endometrial carcinoma cell xenograft growth through increased cell proliferation and survival (11–13) in addition to stimulating carcinoma cell epithelial-mesenchymal transition and invasion (11). Mammary carcinoma cell hGH expression also enhances tumor angiogenesis in a vascular endothelial growth factor-A-dependent manner (11).

The clinicopathologic and prognostic associations of hPRL expression in mammary carcinoma (14) and endometrial carcinoma (15, 16) have been reported. We and others have previously reported the presence of hGH mRNA or protein in human mammary (17–19) and endometrial (20) normal tissue and carcinoma. However, these studies used small sample numbers with inconclusive methodology and results. It has recently been reported that the level of intratumoral GH in mammary carcinoma of dogs can be used to clearly delineate those animals which will survive the disease (21). Furthermore, serum levels of hGH have recently been demonstrated to be significantly associated with endometrial carcinoma (15). In addition to literature evidence supporting a causal role for hGH in mammary and endometrial carcinoma (1–5), these tumors also exhibit similar etiology and responsiveness to estrogenic stimuli. Thus, there is an acute need for a systematic study of the potential association of tumor expression of hGH with clinicopathological features and survival outcome of patients with mammary and endometrial carcinoma.

Herein we report the association of hGH and hPRL expression with specific histopathological features and survival outcomes for patients with mammary or endometrial carcinoma.

Patients and Methods

Patients and specimens

General

The institutional review board approved the protocol for the use of patient samples in this study and informed consent was obtained from all patients and control subjects in accordance with the Declaration of Helsinki. All samples used were from Han Chinese patients. The samples used were sourced from patients with histopathological diagnoses made between 2001and 2006, which permitted us to examine 5-yr survival outcomes for these cohorts. Patients had no previous diagnosis of carcinoma, pituitary adenoma, or obesity (body mass index <28 kg/m^2), no distant metastasis at time of diagnosis, and no evidence of recurrent disease within one month after primary surgery. Patients who had undergone chemotherapy or radiation therapy before surgery were also excluded from this study.

Mammary carcinoma

The patient population consisted of 159 consecutive mammary cancer patients and 33 consecutive patients with benign breast disease who underwent surgery at the First Affiliated Hospital of Anhui Medical University (Hefei, Anhui, People's Republic of China) between 2001 and 2002. The details of this cohort have previously been described in detail (22) including the definition of human epidermal growth factor receptor 2 (HER-2) negative as those tumors with low HER-2 expression [negative (−) and positive (+) staining] and HER-2-positive tumors as those with high HER-2 expression (2+ and 2++ staining). Sixty-five cases were estrogen receptor (ER)+, 50 cases were ER+ Progesterone (PR)+, 75 cases were ER-PR- of which 35 cases were ER-PR-HER2+, and 40 cases were ER-PR-HER2-.

Endometrial carcinoma

The patient population consisted of 70 consecutive uterine endometrial cancer patients, 32 endometrial hyperplasia patients, and 38 normal controls who underwent surgery or curettage at the above hospital between 2004 and 2006. The details of this cohort have previously been described in detail (23) except with the current addition of extra patient numbers.

Tissue microarrays (TMA) construction

Paraffin-embedded tumor/normal specimens were obtained from the archive of the Department of Pathology, the First Affiliated Hospital of Anhui Medical University, People's Republic of China. TMA were constructed as previously described (23). Three tissue spots from two different paraffin blocks of the same tumor or normal tissue were included per patient. The spot diameter for the mammary was 1 mm and for the endometrium was 2 mm.

In situ hybridization (ISH)

Digoxin-labeled antisense oligonucleotide probes for hGH and hPRL were obtained from Boshide Biotech Co. (Wuhan, China). The probe sequences were as follows:

hGH, 5′-TTTGACAACGCTATGCTCCGCGCCCATCG-TCTGCA-3′, 5′-CAGAC CTACA GCAAG TTCGA CACAA ACTCA CACAA-3′, and 5′-TACTGCTTCAGGAAGACAT GGACA AGGTCGAGAC-3′; and hPRL, 5′-CACTACATC-CATAACCTCTCCTCAGAAATGTTCAG-3′, 5′-CGATCCT-GGAATGAGCCTCTGTATCATCTGGTCAC-3′ and 5′-CACT-GCCTACGCAGGGATTCACATAAAATCGACAA-3′.

ISH was performed as described previously (24). A number of controls to determine specificity of the *in situ* hybridization were used and included the following: 1) use of labeled sense probes or 2) application of unlabeled antisense probes to sections at 5 molar excess before application of labeled antisense probes or 3) use of hybridization solution without oligonucleotide probes or 4) use of labeled scrambled sense probes.

Immunohistochemistry (IHC)

Immunohistochemical analysis of hGH, hPRL, and human placental lactogen [hPL; also known as human chorionic somatomammotrophin (hCS)] protein expression was performed on TMA sections (4 μm thick) with polyclonal antibodies against hGH, hPRL (Santa Cruz Biotechnologies, Santa Cruz, CA), and hPL (Maixin Biotechnologies, Fuzhou, China) by the peroxidase-conjugated streptavidin complex method (Histostain-SP kit; Zymed, San Francisco, CA) as previously described (22).

Review and scoring

The stained sections were reviewed and scored for expression of hGH, hPRL, and hPL with a light microscope (Olympus American Inc., Melville, NY) independently by two investigators without knowledge of the patient's clinical or histopathological information as previously described (22, 23). The sections were scored on the basis of the staining intensity and the percentage of cells with staining relative to the background: negative (−), having fewer than 10% of the epithelial cells stained positive; positive (+), having more than 10% of the cells stained positive.

Statistical analysis

All statistical analyses were performed using SPSS software system for Windows (version 13.0; SPSS, Chicago, IL). The χ^2 test was used to analyze the difference in the expression levels among different samples. The statistical significance of potential correlations was determined using the χ^2 test. Spearman's correlation coefficient was calculated to evaluate the relationships between the expression of hGH and hPRL mRNA and hGH, hPRL, or hPL protein. Kaplan-Meier curves were constructed to determine patient relapse-free survival (RFS) and overall survival (OS) rates. Cox regression analysis was performed to determine the association of hGH, hPRL, and hPL expression to the risk of relapse and death. The statistical differences in survival among subgroups were compared using the log-rank test. $P < 0.05$ was considered statistically significant.

Results

Specificity of ISH and IHC

Digoxigenin-labeled antisense oligonucleotide probes specific to hGH or hPRL mRNA were used for ISH. A series of controls were used as negative control for ISH to determine specificity as detailed in materials and methods. hGH and hPL (hCS) are located within the same gene cluster on chromosome 17q23, are thought to have evolved from a common ancestral gene by duplication during evolution, and share considerable nucleic acid sequence homology (25). It is therefore possible that the antisense oligonucleotide probes to hGH could potentially also recognize hPL mRNA. Polyclonal antibodies (pAb) specific to hGH, hPL or hPRL were therefore used to detect the translated hGH, hPRL, and hPL proteins, respectively, in mammary and endometrial TMA. The IHC data were subsequently used for analysis of Spearman's correlations between hGH or hPRL mRNA expression detected by ISH and hGH, hPRL or hPL protein expression detected by IHC (see below). Each pAb specifically recognized its recombinant cognate protein as determined by native PAGE (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Preabsorption of each specific pAb with 10 M excess of the different recombinant proteins (hGH, hPL, or hPRL) demonstrated loss of immunoreactivity on tissue sections only when the specific pAb was preabsorped with its cognate protein (Supplemental Fig. 2).

Further evidence of specificity of the differential hormone expression was provided by analyses of Spearman's correlations between hGH mRNA or hPRL mRNA expression and hGH, hPRL, or hPL protein expression as derived from the mammary and endometrial TMA (Supplemental Table 1). A highly significant correlation was observed between the expression of hGH mRNA and hGH protein and between hPRL mRNA and hPRL protein in mammary (correlation coefficients: $r_s = 0.755$, $P < 0.001$; and $r_s = 0.780$, $P < 0.001$, respectively) and in endometrial specimens ($r_s = 0.752$, $P < 0.001$; and $r_s = 0.840$, $P < 0.001$, respectively). We also observed a lower but significant correlation between the expression of hGH mRNA and hPRL protein and between hPRL mRNA and hGH protein in mammary (correlation coefficients: $r_s = 0.234$, $P = 0.001$; and $r_s = 0.239$, $P < 0.001$, respectively) and in endometrial specimens ($r_s = 0.397$, $P < 0.001$; and $r_s = 0.431$, $P < 0.001$, respectively). However, this correlation is due to significant coexpression of both hormones in a significant number of samples. Indeed, no association between the expression of tumor hGH mRNA and hPRL protein or between the expression of tumor hPRL mRNA and hGH protein was observed by χ^2 analysis (all $P > 0.05$, Supplemental Table 2). No significant Spearman's correlation was observed between hGH mRNA and hPL protein in either mammary or endometrial specimens. A small but significant Spearman's correlation ($r_s = 0.176$ and 0.150, both $P < 0.05$) was observed between expression of hGH and hPL protein in mammary and endometrial specimens, respectively.

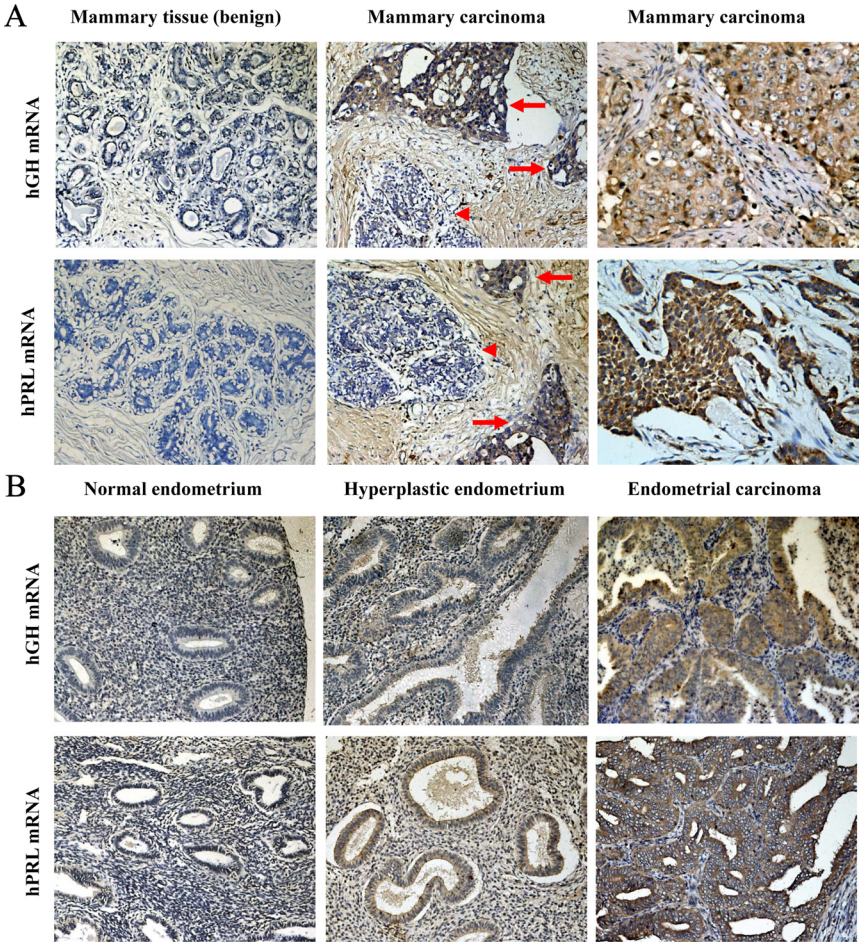

FIG. 1. *In situ* hybridization analysis of hGH or hPRL mRNA expression in mammary and endometrial normal tissue and carcinoma. A, Normal mammary tissue and mammary carcinoma. *Left panels*, Low expression of hGH and hPRL mRNA in normal mammary tissue derived from patients with benign mammary disease. *Center panels*, A sample of mammary carcinoma, which contains both normal tissue (*arrowheads*) and carcinoma (*arrows*). Note that the carcinoma is positive for hGH and hPRL mRNA but that the normal adjacent tissue is negative for the transcripts of both hormones. *Right panels*, High expression of hGH and hPRL mRNA in mammary carcinoma. B, Normal and hyperplastic endometrium and endometrial carcinoma. *Left panels*, Low expression of hGH and hPRL mRNA in normal endometrium. *Center panels*, Moderate expression of hGH and hPRL mRNA in hyperplastic endometrium. *Right panels*, High expression of hGH and hPRL mRNA in endometrial carcinoma. All images are counterstained with hematoxylin. Micrographs were captured at ×200 magnification.

Expression of hGH, hPRL, and hPL in mammary and endometrial normal tissue and carcinoma

We used ISH to detect hGH and hPRL mRNA in mammary and endometrial tissue specimens (Fig. 1). As shown in Supplemental Table 3, 52.8 and 67.9% of mammary carcinoma specimens were positive for hGH and hPRL mRNA, respectively, whereas only 30.3 and 27.3% of normal mammary tissue from patients with benign disease were positive for hGH or hPRL mRNA, respectively ($P = 0.018$ and $P < 0.001$). Examples of hGH and hPRL mRNA positive normal mammary tissue are provided in Supplemental Fig. 3. hGH mRNA expression was observed in normal epithelial cells of mammary ducts and acini with stromal cells not infrequently also positive for hGH mRNA. Infrequent stromal localization of hPRL mRNA was observed. In mammary carcinoma, hGH mRNA was predominantly localized to carcinoma cells (Fig. 1A) with both stromal cells and endothelial cells of blood vessels also frequently positive for hGH mRNA. In general, the expression of hGH mRNA in stromal and endothelial cells was positively correlated to the expression in epithelial cells in the same specimen. Again, in mammary carcinoma, infrequent localization of hPRL was observed in stromal cells.

A similar significantly increased percentage expression of both hGH and hPRL mRNA was observed in endometrial carcinoma compared with normal or hyperplastic endometrial tissues ($P = 0.013$ and $P < 0.001$, respectively) (Supplemental Table 3). In the normal cycling endometrium, the expression of hGH mRNA was significantly higher in the luteal compared with the follicular phase ($P = 0.036$) (Supplemental Table 4). However, no significant difference between phases was observed for the expression of hPRL mRNA ($P > 0.05$, Supplemental Table 4). In normal endometrial tissue, the expression of hGH and PRL mRNA was detected in epithelial cells of the glands and frequently in stromal cells (Supplemental Fig. 3). Similar epithelial and stromal localization was observed in endometrial hyperplasia and carcinoma (Fig. 1B). Endothelial cells were occasionally positive for hGH mRNA but infrequently positive for hPRL mRNA.

hGH and hPRL protein expression in the normal tissue and carcinoma from mammary gland and endometrium exhibited similar (Fig. 2, A and B, and Supplemental Fig. 3) but not identical patterns as observed with the respective mRNA. Such discrepancies may result from technical or processing issues, from differential sensitivity of ISH *vs.* IHC, that hGH and hPRL are secreted proteins, which may affect cellular retention localization or that tumor localized hGH and hPRL protein may represent pituitary derived hormone sequestered by the tumor. In that respect, hGH and hPRL mRNA should be considered a more reliable measure of tumor expression of the hormones than localization of the protein. hPL protein expression was not significantly different between mammary carci-

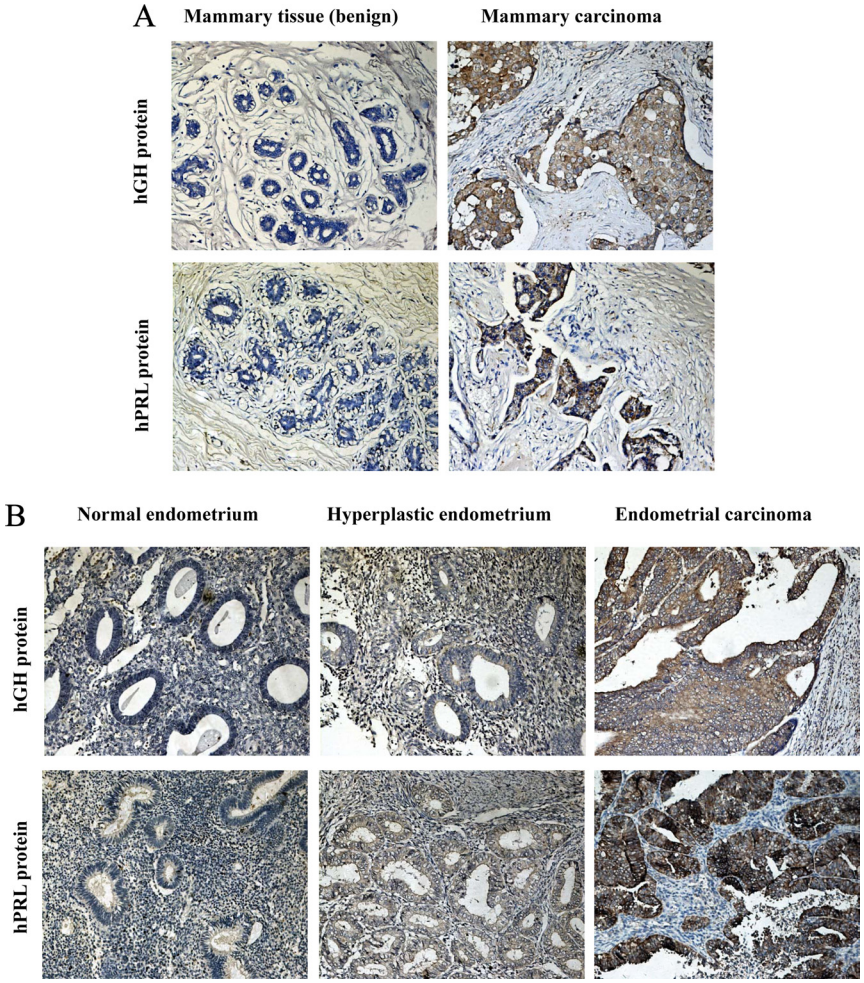

FIG. 2. Immunohistochemical analysis of hGH or hPRL protein expression in mammary and endometrial normal tissue and carcinoma. A, Normal mammary tissue. *Left panels*, Low expression of hGH and hPRL protein in normal mammary tissue derived from patients with benign mammary disease. *Right panels*, High expression of hGH and hPRL protein in mammary carcinoma. B, Normal and hyperplastic endometrium and endometrial carcinoma. *Left panels*, Low expression of hGH and hPRL protein in normal endometrium. *Center panels*, Moderate expression of hGH and hPRL protein in hyperplastic endometrium. *Right panels*, High expression of hGH and hPRL protein in endometrial carcinoma. All images are counterstained with hematoxylin. Micrographs were captured at ×200 magnification.

noma and benign mammary disease ($P = 0.873$) or between endometrial carcinoma and normal or hyperplastic endometrium ($P = 0.112$) (Supplemental Table 3 and Supplemental Fig. 4). hGH, hPRL, and hPL proteins were predominantly expressed in the cytoplasm of normal mammary and endometrial epithelial cells and in carcinoma cells (Fig. 2 and Supplemental Fig. 4). Nuclear localization of the hormones was also observed in accord with previous published reports of nuclear localized hGH and hPRL (26, 27).

Correlation between expression of hGH, hPRL, and hPL and histopathological features of mammary and endometrial carcinoma

We examined for any potential association of the expression of hGH, hPRL, or hPL with the clinicopathological features of mammary and endometrial carcinoma. As shown in Table 1, hGH mRNA expression was positively associated with lymph node metastasis ($P = 0.002$), higher clinical stage ($P = 0.002$), and HER-2 positivity ($P = 0.004$) in mammary carcinoma. hGH protein expression was associated with higher clinical stage ($P = 0.047$), HER-2 positivity ($P = 0.001$), and Ki67 labeling index ($P = 0.002$) in mammary carcinoma. The expression of both hPRL mRNA and protein was associated with lymph node metastasis ($P < 0.001$ and 0.001), higher tumor grade ($P = 0.002$ and 0.015), and higher clinical stage ($P = 0.008$ and $P = 0.046$). The expression of hPL protein was significantly correlated only with the age of patient with mammary carcinoma ($P = 0.009$) and HER-2 positivity ($P = 0.019$). No significant association was observed between the expression of hGH, hPRL, or hPL and the expression of estrogen or progesterone receptors in mammary carcinoma.

In endometrial carcinoma (Table 2), the expression of hGH mRNA was significantly associated with higher tumor grade ($P = 0.004$) and myometrial invasion ($P = 0.006$). hGH protein expression was significantly associated with tumor International Federation of Gynecology and Obstetrics (FIGO) grade ($P = 0.001$), myometrial invasion ($P = 0.025$), and ovarian metastasis ($P = 0.031$). Both the mRNA and protein expression of hPRL was significantly associated with tumor FIGO stage ($P = 0.007$ and $P = 0.009$) and myometrial invasion ($P = 0.011$ and $P = 0.007$). The expression of hPL protein was significantly associated with patient age ($P = 0.037$) and high FIGO grade ($P = 0.009$).

Correlation between hGH, hPRL, or hPL expression and patient survival

To determine whether tumor hGH expression is associated with RFS and OS rates in patients with mammary carcinoma, we first performed Kaplan-Meier analyses on the patient cohort (Fig. 3). As observed in Table 3, patients whose primary tumors did not express hGH mRNA or protein had a mean 5-yr RFS rate of 66.7 and 69.2%,

TABLE 1. Association of tumor hGH or hPRL mRNA and tumor hGH, hPRL, or hPL protein expression with clinicopathological parameters of patients with mammary carcinoma

		hGH-positive expression, n (%)				hPRL-positive expression, n (%)					hPL-positive expression, n (%)		
Parameter	n	mRNA	*P*	Protein	*P*	n	mRNA	*P*	Protein	*P*	n	Protein	*P*
Age (yr)													
≤35	16	9 (56.3)	0.502	11 (68.8)	0.648	16	11 (68.8)	0.623	10 (62.5)	0.144	16	15 (93.8)	**0.009**
35–55	92	45 (48.9)		52 (56.5)		92	65 (70.7)		71 (77.2)		88	55 (62.5)	
>55	51	30 (58.8)		29 (56.9)		51	32 (62.7)		29 (56.9)		49	25 (51.0)	
Tumor size (cm)													
≤2	13	5 (38.5)	0.492	8 (61.5)	0.415	13	9 (62.9)	0.688	10 (76.9)	0.775	12	8 (66.7)	0.457
2–5	115	61 (53.0)		63 (54.8)		115	76 (66.1)		78 (67.8)		110	65 (59.1)	
>5	31	18 (58.1)		21 (67.7)		31	23 (74.2)		22 (71.0)		31	22 (71.0)	
Lymph node metastasis													
0	55	20 (36.4)	**0.002**	28 (50.9)	0.332	55	28 (50.9)	**<0.001**	30 (54.5)	**<0.001**	53	33 (62.3)	0.591
1–3	55	29 (52.7)		32 (58.2)		55	36 (65.5)		37 (67.3)		54	31 (57.4)	
>3	49	35 (71.4)		32 (65.3)		49	44 (89.8)		43 (87.8)		46	31 (67.4)	
Histology													
Ductual	154	81 (52.6)	0.887	89 (57.8)	0.929	154	104 (67.5)	0.423	107 (69.5)	0.44	148	92 (62.2)	0.321
Lobular	2	1 (50.0)		1 (50.0)		2	1 (50.0)		1 (50.0)		2	2 (100.0)	
Other types	3	2 (66.7)		2 (66.7)		3	3 (100.0)		3 (100.0)		3	1 (33.3)	
Grade													
I	13	3 (23.1)	0.079	7 (53.8)	0.802	13	4 (30.8)	**0.002**	6 (46.2)	**0.015**	13	10 (76.9)	0.155
II	102	56 (54.9)		61 (59.8)		102	68 (66.7)		67 (65.7)		97	63 (64.9)	
III	44	25 (56.8)		24 (54.5)		44	36 (81.8)		37 (84.1)		43	22 (51.2)	
Stage													
I–II	85	35 (41.2)	**0.002**	43 (50.6)	**0.047**	85	50 (58.8)	**0.008**	53 (62.4)	**0.046**	83	46 (55.4)	0.064
III–IV	74	49 (66.2)		49 (66.2)		74	58 (78.4)		57 (77.0)		70	49 (70.0)	
Estrogen receptor status													
−	94	53 (56.4)	0.28	57 (60.6)	0.394	94	61 (64.9)	0.325	62 (66.0)	0.29	91	59 (64.8)	0.397
+	65	31 (47.7)		35 (53.8)		65	47 (72.3)		48 (73.8)		62	36 (58.1)	
Progesterone receptor status													
−	90	50 (55.6)	0.432	55 (61.1)	0.343	90	61 (67.8)	0.964	62 (68.9)	0.927	87	56 (64.4)	0.505
+	69	34 (49.3)		37 (53.6)		69	47 (68.1)		48 (69.6)		66	39 (59.1)	
HER-2													
Low	107	48 (44.9)	**0.004**	52 (48.6)	**0.001**	107	74 (69.2)	0.632	77 (72.0)	0.276	104	58 (55.8)	**0.019**
High	52	36 (69.2)		40 (76.9)		52	34 (65.4)		33 (63.5)		49	37 (75.5)	
Ki67													
+	29	10 (34.5)	0.137	9 (31.0)	**0.002**	29	22 (75.9)	0.541	19 (65.5)	0.93	28	17 (60.7)	0.567
++	37	19 (51.40)		19 (51.4)		37	24 (64.9)		27 (73.0)		36	20 (55.6)	
+++	52	30 (57.7)		34 (65.4)		52	37 (71.2)		36 (69.2)		49	37 (75.5)	
++++	41	25 (61.0)		30 (73.2)		41	25 (61.0)		28 (68.3)		40	27 (67.5)	

Values in *bold* are significant ($P < 0.05$).

respectively, whereas patients with tumors expressing hGH mRNA or protein both exhibited a mean 5-yr RFS rate of 50% ($P = 0.041$ and $P = 0.016$). Patients whose tumors were positive for expression of hGH mRNA or protein, respectively, exhibited a lower 5-yr OS rate than patients whose tumors were negative for hGH ($P = 0.024$ and $P = 0.049$, Table 3). Similarly, patients whose tumors express hPRL mRNA or protein also exhibited a lower RFS rate compared with patients whose tumor was negative for hPRL mRNA or protein expression ($P = 0.036$ and $P = 0.009$, Table 3). Patients whose tumors express hPRL mRNA, but not hPRL protein, exhibited a significantly lower OS compared with patients whose tumors were negative for hPRL mRNA or protein, respectively ($P = 0.025$). No significant association was observed between tumor hPL protein expression and patient RFS or OS (both $P > 0.05$). Multivariate analysis of the adjusted odds ratios for death of patients with mammary carcinoma were concordantly significantly elevated in those patients whose tumors expressed hGH or hPRL mRNA. The adjusted odds ratios are presented in Supplemental Table 5.

We next divided the mammary carcinoma cohort into four groups; those whose tumors were negative, those with low expression, those with moderate expression, and those with high expression of hGH or hPRL mRNA or protein, respectively. Kaplan-Meier analysis of RFS and OS of patients with low tumor expression of either hGH or hPRL were not significantly different from those patients whose tumors did not express either hGH or hPRL (see Supplemental Table 6). A clear and significant decrease in RFS and OS was observed in those patients with moderate through high tumor expression of hGH or hPRL, respectively. Higher expression of hGH or hPRL are therefore significantly associated with lower RFS and OS.

The RFS and OS of patients whose tumors were negative for both hGH mRNA and hPRL mRNA was signifi-

TABLE 2. Association of tumor hGH or hPRL mRNA and tumor hGH, hPRL, or hPL protein expression with clinicopathological parameters of patients with endometrial carcinoma

	hGH-positive expression, n (%)					hPRL-positive expression, n (%)					hPL-positive expression, n (%)		
Parameter	**n**	**mRNA**	***P***	**Protein**	***P***	**n**	**mRNA**	***P***	**Protein**	***P***	**n**	**Protein**	***P***
Age (yr)													
<60	57	27 (47.4)	0.155	31 (54.4)	0.137	57	31 (54.4)	0.639	32 (56.1)	0.723	53	39 (73.6)	**0.037**
≥60	13	9 (69.2)		10 (76.9)		13	8 (61.5)		8 (61.5)		13	13 (100)	
Menopausal status													
Premenopausal	17	7 (41.2)	0.331	9 (52.9)	0.588	17	12 (70.6)	0.156	12 (70.6)	0.198	16	14 (87.5)	0.327
Postmenopausal	53	29 (54.7)		32 (60.4)		53	27 (50.9)		28 (52.8)		50	38 (76.0)	
FIGO stage													
I + II	62	30 (48.4)	0.156	35 (56.5)	0.316	62	31 (50.0)	**0.007**	32 (51.6)	**0.009**	58	47 (81.0)	0.229
II + IV	8	6 (75.0)		6 (75.0)		8	8 (100.0)		8 (100.0)		8	5 (62.5)	
FIGO grade													
1	29	9 (31.0)	**0.004**	10 (34.5)	**0.001**	29	13 (44.8)	0.123	14 (48.3)	0.207	27	17 (63.0)	**0.009**
2 + 3	41	27 (65.9)		31 (75.6)		41	26 (63.4)		26 (63.4)		39	35 (89.7)	
Lymph node metastasis													
−	67	33 (49.3)	0.085	39 (58.2)	0.771	67	36 (53.7)	0.114	37 (55.2)	0.125	63	51 (81.0)	0.05
+	3	3 (100.0)		2 (66.7)		3	3 (100.0)		3 (100.0)		3	1 (33.3)	
Myometrial invasion													
No	15	3 (20.0)	**0.006**	5 (33.3)	**0.025**	15	4 (26.7)	**0.011**	4 (26.7)	**0.007**	15	10 (66.7)	0.191
Yes	55	33 (60.0)		36 (65.5)		55	35 (63.6)		36 (65.5)		51	42 (82.45)	
Cervical involvement													
Negative	64	33 (51.6)	0.942	39 (60.9)	0.189	64	35 (54.7)	0.572	36 (56.3)	0.622	60	48 (80.0)	0.446
Positive	6	3 (50.0)		2 (33.3)		6	4 (66.7)		4 (66.7)		6	4 (66.7)	
Ovarian metastasis													
Negative	64	31 (48.4)	0.102	35 (54.7)	**0.031**	64	34 (53.1)	0.154	35 (54.7)	0.175	60	47 (78.3)	0.775
Positive	6	5 (83.3)		6 (100)		6	5 (83.3)		5 (83.3)		6	5 (83.3)	
Estrogen receptor status													
−	16	8 (50.0)	0.896	8 (50.0)	0.428	16	6 (37.5)	0.095	9 (56.3)	0.935	16	12 (75.0)	0.67
+	54	28 (51.9)		33 (61.1)		54	33 (61.1)		31 (57.4)		50	40 (80.0)	
Progesterone receptor status													
−	15	9 (60.0)	0.454	7 (46.7)	0.291	15	10 (66.7)	0.335	11 (73.3)	0.153	15	10 (66.7)	0.191
+	55	27 (49.1)		34 (61.8)		55	29 (52.7)		29 (52.7)		51	42 (82.4)	

Values in *bold* are significant ($P < 0.05$).

cantly higher than patients whose tumors were positive for mRNA expression of either hGH or hPRL (Table 3). Approximately 40% of mammary carcinomas were positive for both hGH and hPRL mRNA (Supplemental Table 2). The RFS and OS rates for patients whose tumors were negative for the mRNA for both hormones were greatly and significantly higher compared with those patients whose tumors were both hGH mRNA and hPRL mRNA positive ($P = 0.005$ and $P = 0.007$, respectively) (Fig. 3 and Table 3) or those patients whose tumor was either hGH mRNA positive or hPRL mRNA positive alone.

Given the association of hGH expression with HER-2 positivity observed above, we examined for a potential association of hGH or hPRL expression with RFS or OS in the subgroups of patients with tumors with either HER-2 low or HER-2 high expression. Although the expression of hGH or PRL mRNA in patients with HER-2-low tumors tended to correlate with RFS and OS, only the expression of hGH protein was significantly correlated with a shorter RFS ($P = 0.024$, Supplemental Table 7). In patients with HER-2-high tumors, no such tendency or significant correlation with RFS or OS existed for hGH mRNA or protein ($P > 0.05$). However, hPRL mRNA expression was significantly correlated with RFS ($P = 0.035$) (hPRL protein approached but did not reach significance), and hPRL protein was significantly correlated with OS ($P = 0.04$) in HER-2-high patients (hPRL mRNA approached but did not reach significance).

Similarly for endometrial carcinoma, Kaplan-Meier analyses demonstrated that the expression of either hGH or hPRL mRNA or protein predicted poor RFS and OS, compared with patients whose tumors did not express

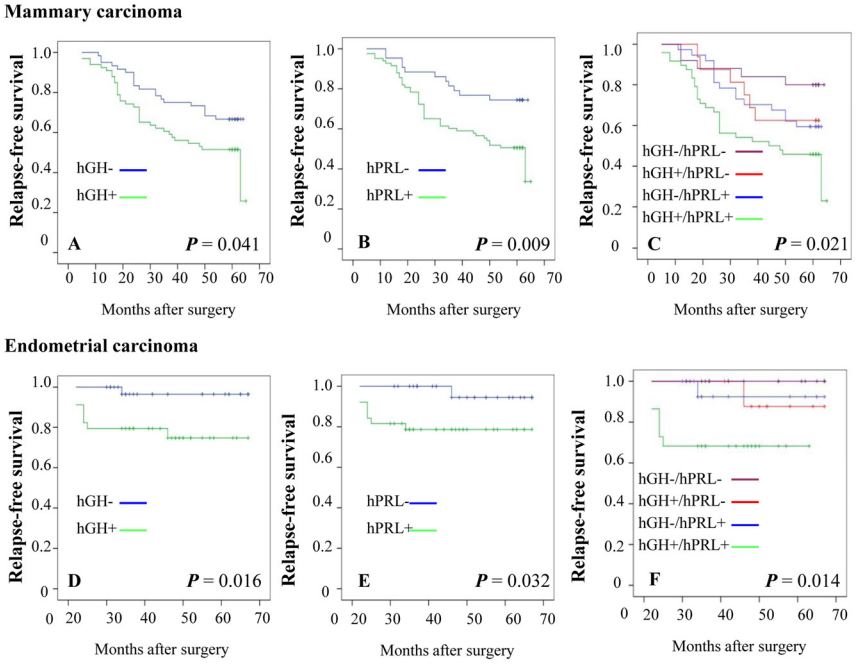

FIG. 3. Kaplan-Meier analysis of the significance of expression of tumor hGH mRNA or hPRL mRNA or both on RFS of patients with mammary and endometrial carcinoma.

hGH or hPRL, respectively (all $P < 0.05$). No significant association was observed between hPL protein expression and endometrial carcinoma patient RFS or OS rate (both $P > 0.05$). The adjusted odds ratios for death of patients with endometrial carcinoma were significantly elevated in those patients whose tumors expressed hGH mRNA but not hPRL mRNA or protein. The adjusted odds ratios are presented in Supplemental Table 6.

Similarly as for mammary carcinoma, we divided the endometrial carcinoma cohort into four groups: those whose tumors were negative, those with low expression, those with moderate expression, and those with high expression of hGH or hPRL mRNA or protein, respectively. Kaplan-Meier analysis of RFS and OS of patients with low tumor expression of either hGH or hPRL mRNA or protein was not significantly different from those patients whose tumors did not express either hGH or hPRL mRNA or protein (see Supplemental Table 7). A clear and significant decrease in RFS and OS was observed in those patients with moderate through high tumor expression of hGH or hPRL mRNA or protein, respectively. Higher tumor expression of hGH or hPRL mRNA or protein are therefore significantly associated with lower RFS and OS in patients with endometrial carcinoma.

Approximately 25% of endometrial carcinoma were negative for both hGH mRNA and hPRL mRNA, and 20% were negative for both hGH and hPRL protein (Supplemental Table 2). Interestingly, these patients whose tumor was negative for both hGH and hPRL mRNA or protein exhibited 100% RFS and OS in the 5-yr follow-up (Table 3). Approximately 33% of endometrial carcinoma were both hGH mRNA positive and hPRL mRNA positive, and this subgroup of patients had a worse survival than those with either hGH mRNA expression or hPRL mRNA expression alone (Supplemental Table 6).

Discussion

Herein we have documented a significant association of tumor hGH and hPRL expression with histopathological features of mammary and endometrial carcinoma and with poor survival of patients with those tumors. This study therefore provides clear support for the clinical relevance of previously published work reporting an autocrine/paracrine role of hGH and hPRL in mammary and endometrial carcinoma cells (1–5). The association of hGH and hPRL expression with tumor histopathological features promoting poor survival outcome in both mam-

TABLE 3. Association of tumor hGH and hPRL mRNA and hGH, hPRL, or hPL protein expression with 5-yr RFS and OS in patients with mammary and endometrial carcinoma

	Mammary carcinoma								Endometrial carcinoma							
	RFS (%)				OS (%)				RFS (%)				OS (%)			
	mRNA	*P*	Protein	*P*	mRNA	*P*	Protein	*P*	mRNA	*P*	Protein	*P*	mRNA	*P*	Protein	*P*
hGH−/hGH+	66.7/50.0	**0.041**	69.2/50.0	**0.016**	73.3/54.5	**0.024**	73.1/56.8	**0.049**	97.0/76.5	**0.016**	96.6/78.9	**0.035**	97.0/76.5	**0.017**	96.6/78.9	**0.028**
hPRL−/hPRL+	74.4/49.4	**0.009**	71.4/51.2	**0.036**	76.7/56.6	**0.025**	73.8/58.3	0.082	96.6/78.9	**0.032**	96.4/79.5	**0.039**	96.6/78.9	**0.023**	96.4/79.5	**0.028**
hPL−/hPL+			56.3/59.5	0.815			64.6/63.5	0.76			87.8/78.6	0.354			87.8/78.6	0.391
hGH-hPRL−/hGH+	80.0/50.6	**0.016**	77.3/50.0	**0.037**	84.0/57.6	**0.025**	77.3/56.8	0.094	100.0/78.9	**0.046**	100.0/78.9	**0.039**	100.0/78.9	**0.041**	100.0/78.9	**0.041**
hGH- hPRL−/hPRL+	80.0/48.4	**0.011**	77.3/52.3	0.053	84.0/53.1	**0.011**	77.3/59.3	0.133	100.0/76.5	**0.034**	100.0/78.4	**0.047**	100.0/76.5	**0.032**	100.0/78.4	**0.034**
hGH−hPRL−hPL−/hPL+			87.5/76.9	0.511			87.5/76.9	0.511			100.0/100.0	1			100.0/100.0	1
hGH-hPRL−/hGH+ hPRL+	80.0/43.8	**0.005**	77.3/46.4	**0.021**	84.0/50.0	**0.007**	77.3/53.6	0.062	100.0/68.2	**0.012**	100.0/70.8	**0.029**	100.0/68.2	**0.013**	100.0/70.8	**0.022**

Values in *bold* are significant ($P < 0.05$).

mary and endometrial carcinoma is also consistent with the published biological effects of these hormones acting in an autocrine or paracrine manner (1–5). For example, in this study, hGH expression was highly and significantly associated with myometrial invasion in endometrial carcinoma. Previous work has demonstrated that forced expression of hGH in endometrial carcinoma cells results in a highly invasive cell phenotype with *in vivo* tumor cell invasion (13). Myometrial invasion itself is a poor prognostic factor for patients with endometrial carcinoma (13). Similarly, the association of hGH expression with the Ki67 labeling index in mammary carcinoma is consistent with the published proliferative actions of autocrine hGH in human mammary carcinoma cells (2, 9). Furthermore, consistent with our work herein, and while this work was in progress, it was reported (21) that intratumoral GH levels in dogs with mammary carcinoma were of prognostic survival value for these animals.

Herein, we have described an association of both hGH and hPL expression with HER-2 positivity. The *HER-2* gene is amplified in approximately 20% of human breast cancers, in which it is associated with aggressive disease and early development of metastasis. The HER-2 status of mammary carcinoma is clinically relevant and functions as an effective biomarker of patient prognosis and therapeutic susceptibility to trastuzumab (Herceptin), a Food and Drug Administration-approved humanized monoclonal antibody targeting HER-2 (28). In this regard it is interesting that frequent coamplification of two different regions on chromosome 17q has been reported (25) with HER-2 at 17q21. The 66.5-kb hGH and placental lactogen gene cluster is located at chromosome 17q23, within the frequently amplified 17q22-q24 region (25), and contains the genes for *GH1*, *CSHP1*, *CSH1*, *GH2*, and *CSH2* (29). The *CSH* (hPL) genes were reported to be amplified in 22% of mammary carcinomas and both the hPL and *HER-2* genes were frequently and significantly coamplified (25). It has therefore also been suggested (25), and given the association of hGH expression with HER-2 status observed in this study, that it is expected that the hGH genes would also be coamplified with hPL. In contrast, the hPRL gene is located on chromosome 6, and concordantly, no association of hPRL expression with HER-2 status was observed in this study. However, previous reports have detailed extensive histopathological association and functional interactions between hPRL and HER-2 (30, 31). For example, mammary carcinomas with coexpression of hPRL and HER-2 exhibit higher proliferative and metastatic activity than carcinomas with expression of either alone (30). PRL stimulates the Janus kinase-2-dependent phosphorylation of HER-2, association of HER-2 with growth factor receptor-bound protein-2, and activation of MAPK in mammary carcinoma cells (30). Similarly, hGH has also been reported to stimulate phosphorylation of the epidermal growth factor receptor and its use as a signaling adaptor (32). hPL expression was also detected in mammary cancers with no amplification of the hPL genes, suggestive that amplification was not the only mechanism producing increased expression of hPL in mammary cancer (27). Similarly, we observed herein that hPL and hGH are also expressed in HER-2 low tumors. Recent genetic studies (27) have given importance to amplified oncogenes in establishing the carcinoma cell phenotype (33). That the hGH gene cluster appears to be frequently amplified in mammary cancer and that hGH is significantly increased in expression in mammary carcinoma and associated with survival outcome are indicative of a pivotal role for hGH in mammary carcinoma.

We observed independent and synergistic effects of hGH and hPRL on survival outcomes in mammary and endometrial carcinoma. The most favorable survival outcome was observed in patients whose tumors, either mammary or endometrial, were both hGH and hPRL negative. This observation is concordant with extensive similarities and redundancies in signal transduction pathways used by these hormones (33–35) and reported autocrine effects of these hormones in mammary and endometrial carcinoma cells (9, 13, 36, 37). hGH is able to bind to both the hGH and hPRL receptors (38), and recent reports also suggest hGH-hPRL receptor heterodimers may be formed in human mammary carcinoma cells (39). A single point mutation at G120 in hGH is therefore able to generate an antagonist to hGH, hPRL, and hPL, whereas the analogous point mutation in hPRL produces only a hPRL-specific antagonist (37). There may, however, be alternative interacting proteins for these hormones. Indeed, these hormones have been demonstrated to bind to lysyl oxidase in order of affinity hPL greater than hGH greater than hPRL (40). hPL was also demonstrated to functionally synergize with lysyl oxidase to increase mammary carcinoma cell proliferation and migration. Clevenger and colleagues (26) have furthermore previously demonstrated that both hPRL and hGH physically and functionally interact with cyclophilin B. It is possible that the interaction of hGH and hPRL with alternative proteins of functional significance may use different regions of the hormones required for interaction with the classic hGH and hPRL receptors. Regardless of the promiscuity of these ligands, antagonists such as hGH-G120R, able to effect dual inhibition of both hGH and hPRL, would presumably not only be more functionally efficacious to inhibit tumor growth but also more cost effective for clinical use. Indeed, combined inhibition of hGH and hPRL with specific antagonists elicits more

profound inhibitory effects on mammary carcinoma cell signaling pathways than does either antagonist alone (39). Such dual-inhibitory strategies would presumably also reduce the potential for acquisition of tumor resistance to single administration of either specific hGH or hPRL inhibitors.

In summary, we have observed that expression of both hGH and hPRL in mammary and endometrial carcinoma predicts a large and significant difference in survival outcome for patients with these tumors. In addition, hGH and hPRL also function as endocrine hormones, with hGH the major regulator of both peripheral and local IGF-I expression (5). Indeed, animal and primate studies have demonstrated a pivotal role of endocrine GH in tumor initiation and progression (for review see Refs. 2 and 5). The survival differences observed herein are clearly independent of pituitary-derived hGH and consequent hGH-dependent hepatic IGF-I expression or pituitary-derived hPRL. It is therefore logical speculation that combined systemic inhibition of hGH and hPRL will produce greater survival advantage to patients than can be inferred from the tumor expression data herein. The functional responses of both hGH negative and hGH positive primary human mammary carcinoma cells to exogenous hGH (41) lends credence to this notion. The accumulated literature is compelling and must provide impetus for serious consideration of the combined therapeutic inhibition of hGH and hPRL, at least in mammary and endometrial carcinoma.

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Address all correspondence and requests for reprints to: Peter E. Lobie, M.D., Ph.D., Cancer Science Institute of Singapore, National University of Singapore, Centre for Life Sciences, 03-06C, 28 Medical Drive, Singapore 117456. E-mail: csipel@nus.edu.sg; or Tao Zhu, M.D., Ph.D., Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, People's Republic of China. E-mail: zhuttt@gmail.com.

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