Oncogenic Transformation of Human Mammary Epithelial Cells by Autocrine Human Growth Hormone

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Abstract

The human growth hormone (hGH) gene is expressed in the normal human mammary epithelial cell and its expression increases concomitant with the acquisition of proliferative lesions. Herein we demonstrate that autocrine production of hGH in human mammary carcinoma cells dramatically enhances anchorage-independent growth in a Janus kinase 2–dependent manner. Forced expression of the hGH gene in immortalized human mammary epithelial cells increased proliferation, decreased apoptosis, altered the cellular morphology and resulted in oncogenic transformation. Autocrine hGH was therefore sufficient to support anchorage-independent growth of immortalized human mammary epithelial cells and tumour formation in vivo. Moreover, autocrine hGH disrupted normal mammary acinar architecture with luminal filling and deregulated proliferation in three-dimensional epithelial cell culture. Autocrine hGH utilized homeobox A1 to govern the transcriptional program required for autocrine hGH–stimulated oncogenic transformation of human mammary epithelial cells, including transcriptional up-regulation of c-Myc, cyclin D1, and Bcl-2. Forced expression of a single orthotopically expressed wild-type gene is therefore sufficient for oncogenic transformation of the immortalized human mammary epithelial cell.

Introduction

It has been hypothesized that cancer arises from a stepwise accumulation of genetic changes that liberates neoplastic cells from the homeostatic mechanisms that govern normal cell proliferation (1). A contrasting but complementary hypothesis has recently been proposed that deregulation of proliferation, together with a reduction in apoptosis, creates a platform that is both necessary and sufficient for development of cancer (2). Such a hypothesis is reinforced by the fact that many mechanisms that govern the transcriptional program required for autocrine hGH administration results in marked hyperplasia of the mammary gland with an increased epithelial proliferation index (13). We have demonstrated the expression of the hGH gene in the normal human mammary gland epithelium (14). Increased epithelial expression of the hGH gene is associated with the acquisition of pathologic proliferation and the highest level of hGH gene expression is observed in metastatic mammary carcinoma cells (14). The autocrine production of hGH in human mammary carcinoma cells has been shown to result in a hyperproliferative state (15) with an aggressive cellular morphology (16).

In the present study, we demonstrate that autocrine production of hGH in immortalized human mammary epithelial cells concomitantly enhances proliferation and offers protection from apoptosis; forming the basis for abnormal mammary acinar morphogenesis, oncogenic transformation, and tumor formation in vivo. Thus, simple forced expression of a single orthotopically expressed wild-type gene is sufficient for oncogenic transformation of the immortalized human mammary epithelial cell.

Materials and Methods

Cell Lines, Cell Culture, and Transfections. The MCF-7, MCF-10A, and NIH-3T3 cell lines were obtained from the American Type Culture Collection. MCF-7-hGH expressing wild-type hGH gene and its cognate control MCF-7-MUT were established as described (15). A detailed description of the characterization of these cell lines has been published previously (15, 16). pcDNA3 vector alone and pcDNA3-hGh vector were stably transfected into NIH-3T3 cells or MCF-10A cells by use of Effectene (Qiagen, Inc., Valencia, CA). Expression of hGH mRNA in the stable cell lines were confirmed by both reverse transcription–PCR (RT-PCR) and ELISA as described previously (15, 16).
5′-Bromo-2′-Deoxyuridine Incorporation Assay and Measurement of Apoptosis. Mitogenesis was directly assayed by measuring the incorporation of bromodeoxyuridine (16). Apoptotic cell death was measured by fluorescent microscopic analysis of cell DNA staining patterns with Hoechst 33258 as previously described (16).

Cell Behavior and Morphogenesis Assays in Matrigel. Tissue culture dishes were coated with Matrigel (BD Bioscience, Franklin Lakes, NJ) at 37°C for 30 minutes before adding 1 × 10^5 vector- or hGH-transfected MCF-10A cells. The behavior of both cell lines was assessed and digitally recorded at 6-hour intervals using an inverted light microscope (Nikon TE200, Nikon, Tokyo, Japan). The three-dimensional culture of MCF-10A cells on basement membrane was done as described (17). Assay medium with 2% Matrigel was replaced every 4 days.

Confocal Laser Scanning Microscopic Analysis and Image Acquisition. Acinar structures were fixed in 2% formalin at room temperature for 25 minutes and permeabilized in 0.3% Triton X-100 in PBS for 10 minutes at 4°C. Except for this minor modification in fixation and permeabilization, the immunostaining of acinar structures was carried out as described (17). Anti-GM130 was purchased from BD Transduction (San Diego, CA), anti-α1 integrin from Chemicon (Temecula, CA), phospho-ERM from Cell Signaling (Beverley, MA), and TO-PRO3 from Molecular Probes (Eugene, OR). Confocal analyses were done using the Bio-Rad MRC1024 confocal microscopy system (Bio-Rad, Hercules, CA).

Reverse Transcription-PCR. Extraction of total RNA and RT-PCR assay were done as described (18). Sequences of the oligonucleotide primers used for RT-PCR are as follows: 5′-AAA GCC CCC CAA GGTG TA-3′ and 5′-TTT TTG GCT CAA GCT GCA-3′ for c-myc; 5′-CCCG TCG GTG TCC TAT CTC AAA-3′ and 5′-CAG CTC CTC CTC CTC CTC TCC-3′ for Cyclin D1; 5′-ATG GCC CAC GGT GGA AGA AC-3′ and 5′-GGCG GTAAG CCG GAG AAG T-3′ for Bcl-2; and 5′-ATG ATA TCG CCG CGC TCG-3′ and 5′-CCG GTG AGG ATC TTC A-3′ for β-Actin.

Soft Agar Colony Formation and Suspension Culture Assays. Anchorage-independent growth assays, including suspension culture, were performed as previously described (19). For soft agar colony formation assay, MCF-7 cell and its derived cell lines were cultured in six-well plates with a 0.35% agar layer with or without 50 mmol/L hGH at 35 ± 0.5 mmol/L Janus-activated kinase (JAK) 2-specific inhibitor AG490 or Src kinase inhibitor PP1 and PP2 (20, 21). The MCF10A-vector and MCF10A-hGH cell soft agar colony formation assays were processed the same as above, except that the medium was changed to DMEM/Ham's F-12 medium containing appropriate components as described (19), and 10^4 cells were seeded to the middle layer of the soft agar. The plates were incubated for 10 days (for MCF-7 cells) or 14 days (for MCF-10A cells), after which the cultures were inspected and photographed.

Luciferase Reporter Assay. Transient transfection was performed with the respective luciferase constructs (18) and other expression constructs as appropriate were transfected in serum-free medium for 48 hours. Results were normalized to the level of appropriate luciferase constructs (18) and other expression constructs as normalized to the level of the respective luciferase constructs. The results were indicated by colony formation in soft agar (Fig. 1A).

Results

Autocrine hGH Production by Mammary Carcinoma Cells Enhances Anchorage-Independent Growth. One characteristic of oncogenically transformed cells is the capacity for anchorage-independent growth (1–3). Autocrine production of hGH in MCF-7 cells dramatically enhanced anchorage-independent growth as indicated by colony formation in soft agar (Fig. 1A) and growth in suspension culture (Fig. 1C). Furthermore, the individual colony size was markedly increased by autocrine hGH (Fig. 1B). Exogenously applied recombinant hGH (50 mmol/L applied daily) did not enhance colony formation in soft agar by MCF-7 cells (Fig. 1A) and, surprisingly, slightly abrogated the effect of autocrine hGH on colony formation. Cellular effects of hGH are primarily mediated by stimulation of JAK2 activity (22). The enhanced colony formation by MCF-7 cells due to autocrine hGH production was completely inhibited by a specific inhibitor of JAK2 (Fig. 1D), whereas the basal level of MCF-7 cell colony formation was not significantly affected by inhibition of JAK2, Src kinase–specific inhibitors PP1 and PP2 (or the inactive structural analogue PP3) did not inhibit autocrine hGH–stimulated colony formation (data not shown). Thus, autocrine hGH enhancement of mammary carcinoma cell anchorage-independent growth is JAK2 dependent.

Autocrine hGH Causes Oncogenic Transformation of Immortalized Human Mammary Epithelial Cells. To determine if forced expression of hGH would result in oncogenic transformation of human mammary epithelium we used the immortalized human mammary epithelial cell line MCF-10A.
MCF-10A cells do not express the hGH gene nor secrete any significant amount of hGH (Fig. 2A). Stable transfection of MCF-10A cells with the complete hGH gene resulted in cellular hGH production and secretion of hGH from the cell (Fig. 2B). The concentration of hGH produced in the cell is similar to that reported for hGH content of human intraductal carcinoma (14, 15). Autocrine production of hGH in this cell line both significantly increased mitogenesis (Fig. 3A) and abrogated apoptotic cell death as a consequence of serum deprivation (Fig. 3B) when compared to vector-transfected control cells. Consequently, autocrine production of hGH by MCF-10A cells resulted in an approximate 4-fold increase in cell number (data not shown). Microscopic evaluation revealed differences in morphology between MCF-10A cells with autocrine production of hGH compared to vector-transfected control cells. Vector-transfected MCF-10A cells displayed an epithelial morphology, with cell to cell interaction, whereas MCF-10A-hGH exhibited a mesenchymal spindle-shaped phenotype with limited intercellular contact (data not shown). MCF-10A-vector cells rapidly organized into spherical colonies when cultured on Matrigel (Fig. 3C). In contrast, MCF-10A-hGH cells cultured on Matrigel adopted a stellate organization (Fig. 3C) that was indistinguishable from the behavior of aggressive mammary carcinoma cell lines such as MDA-MB-231 and MDA-MB-435 (data not shown).

The morphologic changes observed in immortalized mammary epithelial cells due to autocrine production of hGH are reminiscent of oncogenic transformation. Another characteristic of the transformed phenotype is the capacity for anchorage-independent proliferation (3, 9). MCF-10A-hGH cells formed numerous colonies in soft agar, whereas MCF-10A-vector cells were largely ineffective in colonization of soft agar (Fig. 4A and B). Generation of two further stable clone sets of MCF-10A-vector and MCF-10A-hGH confirmed this observation. Application of exogenous hGH did not support soft agar colony formation by MCF-10A-vector cells and paradoxically significantly inhibited the ability of MCF-10A-hGH cells to colonize soft agar (Fig 4B). Use of the JAK2-specific inhibitor AG490 prevented soft agar colony formation by MCF-10A-hGH cells, indicative that oncogenic transformation stimulated by autocrine production of hGH required the activity of JAK2 (Fig. 4C).

**Autocrine Production of hGH Results in the Filling of the Luminal Space in the Mammary Acinus.** When immortalized human mammary epithelial cells (MCF-10A) are cultured *ex vivo* in Matrigel, a source of extracellular matrix akin to basement membrane, acinar structures resembling the *in vivo* morphology of the mammary gland are formed (17, 23, 24). The construction of these acini requires coordinated apoptosis to enable lumen formation and proliferative arrest of the remaining outer layer of mammary epithelial cells (17). In contrast, oncogenically transformed mammary epithelial cells form large, nonpolarized, undifferentiated colonies without lumina when grown in Matrigel (23). We used this *ex vivo* model to examine the effects of autocrine hGH on the architecture of structures formed by immortalized human mammary epithelial cells (24). Three-dimensional acinar structures were generated by plating MCF-10A cells as single cells in Matrigel. Phase-contrast (Fig. 5A) and confocal laser scanning microscopic analyses of acini labeled with TO-PRO3 revealed that...
the acinar units had basally localized nuclei and a hollow lumen (Fig. 5C and E). Localization of a basal surface marker (αv integrin; Fig. 5C), plasma membrane marker (phospho-ERM; Fig. 5E), and apical polarity marker (GM130; Fig. 5C) indicated that the acini formed consisted of polarized mammary epithelial cells. The small number of cells labeled with a proliferation marker (Ki-67; Fig. 5C) was consistent with proliferative arrest observed previously (17, 24). Autocrine production of hGH in immortalized human mammary epithelial cells resulted in the generation of large disorganized multiacinarian structures with filled lumina (Fig. 5B-F), indicative of failure of luminal apoptosis. Autocrine production of hGH in immortalized human mammary epithelial cells also disrupted cellular polarization (Fig. 5C versus D and E versus F) and bypassed the proliferative arrest observed on normal acinar formation (Fig. 5C versus D). Labeling of cells with a lipophilic dye (DiI) and combination with unlabeled cells during the

**Figure 3.** Autocrine production of hGH by immortalized human mammary epithelial cells regulates cell survival, proliferation, and morphology. A, effect of autocrine hGH production by MCF-10A cells on cell cycle progression indicated by nuclear incorporation of bromodeoxyuridine. B, effect of autocrine hGH production by MCF-10A cells on apoptosis induced by serum withdrawal. C, phase-contrast morphology of MCF-10A-vector and MCF-10A-hGH cells cultured on Matrigel.

**Figure 4.** Autocrine hGH stimulation of anchorage-independent growth in immortalized human mammary epithelial cells is JAK2 dependent. A, visualization of soft agar colony formation by MCF-10A-vector and MCF-10A-hGH cells as indicated. B, soft agar colony formation in MCF-10A-hGH or MCF-10A-vector ± 50 nmol/L exogenous hGH added daily. C, effect of inhibition of JAK2 activity with 50 μmol/L AG490 on soft agar colony formation by MCF-10A-vector and MCF-10A-hGH cells. Columns, mean of triplicate determinations; bars, SD. *, P < 0.01.
morphogenesis assay showed that each acinus was derived from a single cell (data not shown).

Autocrine hGH Production in Immortalized Human Mammary Epithelial Cells Confers Tumorigenic Capacity. In vitro analyses of oncogenic transformation are not always concordant with tumorigenic potential in vivo. We therefore implanted both MCF-10A-vector and MCF-10A-hGH cells into the first mammary (axillary) fat pad of intact athymic female mice with use of either PBS or Matrigel as vehicle. Neither cell line produced palpable tumors when injected with PBS. However, MCF-10A-hGH cells with Matrigel injected as vehicle formed large palpable tumors (average size, 306 ± 43 mm³) in the majority of injected animals (8/10), whereas MCF-10A-vector cells did not (0/10; Fig. 6A). The latency of macroscopic tumor appearance was approximately 3 weeks and tumors were harvested 6 weeks after injection. Necropsy revealed that the tumors were attached to the underlying axillary muscle and surrounded by a vascular fibrous capsule (Fig. 6B). Histologically, the neoplastic cells were locally invasive and associated with fibrous connective tissue (Fig. 6C). The cells exhibited moderate cytoplasmic and nuclear pleomorphism and formed a solid mass. Necropsy of female athymic mice given injections of MCF-10A-vector cells failed to identify any growth. Macroscopic and histologic examination of lung and liver failed to identify metastatic extension of the implanted MCF-10A-hGH cells (data not shown). Mammary carcinoma cells derived from the in vivo growth of MCF-10A-hGH cells expressed hGH at the same level as the injected cell, indicative of phenotypic retention (data not shown).

Autocrine hGH Oncogenically Transforms NIH-3T3 Cells. We next wished to determine if oncogenic transformation by autocrine production of hGH was a generalized phenomenon and not particular to the human mammary epithelial cell. NIH-3T3 cells are widely used in studies of oncogenic transformation (1, 25) and are responsive to hGH stimulation (20, 21, 26). We therefore generated stable NIH-3T3 transfectants with autocrine expression of hGH (data not shown). Autocrine production of hGH by NIH-3T3 cells resulted in dramatic foci formation and robust soft agar colony formation (Fig. 7A and B); two indicators of oncogenic transformation of NIH-3T3 cells not observed in vector-transfected control cells. Concordant with this observation, autocrine production of hGH stimulated a dramatic increase in NIH-3T3 cell number compared to the vector-transfected control (Fig. 7C).

Both Proliferative and Cell Survival Gene Transcription Is Required for Oncogenic Transformation by Autocrine hGH. Activation of signaling pathways resulting in oncogenic transformation is associated with transcriptional regulation of genes required for cell cycle progression and cell survival (3, 9). c-Myc and Cyclin D1 are required for mammary epithelial cell cycle progression, whereas Bcl-2 is required for mammary epithelial cell survival (1, 2, 27). c-Myc, cyclin D1, and Bcl-2 have all been reported to be overexpressed in breast cancer biopsies (28). Autocrine hGH increased the transcription and subsequent mRNA level and protein expression of c-Myc, Cyclin D1, and Bcl-2 in human mammary epithelial cells (Fig. 8A-C). Transient transfection of siRNA to c-Myc, Cyclin D1, or Bcl-2 significantly abrogated the...
ability of autocrine hGH to stimulate colony formation in soft agar (Fig. 8E); the extent consistent with the efficiency of transfection (data not shown). Thus, activation of genes required for both proliferation and cell survival is necessary for oncogenic transformation of immortalized human mammary epithelial cells by autocrine hGH.

**HOXA1 Governs the Transcriptional Program of Autocrine hGH Required for Oncogenic Transformation.** We have recently reported that an autocrine hGH–regulated gene, **HOXA1**, is itself a powerful human mammary epithelial oncogene (19). Homeobox genes often govern higher order genetic switches that determine cell fate by coordination of regulatory pathways (29). Construction and transfection of an siRNA to **HOXA1** significantly impaired the ability of autocrine hGH to increase **HOXA1**-mediated transcription (Fig. 8D). Knockdown of **HOXA1** also significantly inhibited the ability of autocrine hGH to stimulate transcription of the c-Myc, Cyclin D1, and Bcl-2 genes (Fig. 8D), indicative that autocrine hGH regulation of these genes is predominantly governed by **HOXA1**. Basal transcription of c-Myc, CyclinD1, and Bcl-2 was not affected by the diminished expression of **HOXA1** (data not shown). Transient transfection of the same **HOXA1** siRNA construct also dramatically abrogated the ability of autocrine hGH to stimulate colony formation by human mammary epithelial cells in soft agar (Fig. 8E). Thus, autocrine hGH is using **HOXA1** to govern the expression of a transcriptional program, resulting in proliferation, cell survival, and ultimately oncogenic transformation of immortalized human mammary epithelial cells.

**Discussion**

We show here that autocrine production of hGH, by itself, is sufficient to oncogenically transform the immortalized human mammary epithelial cell with consequent tumor formation in vivo. This is remarkable given that other oncogenes, such as ras, HER2, TC21, and Cyclin D1 are insufficient to convey tumorigenic potential on MCF-10A cells (19, 30). This is presumably because many mechanisms that drive cell proliferation also possess the potential to trigger or sensitize a cell to apoptosis (2, 3). Established examples include activation of the Ras/Raf pathway and deregulated expression of c-Jun, c-Myc, and E2F transcription factors (2). Moreover, other versions of this so-called antagonistic pleiotropy (2) exist, such as the innate capacities of activated Raf and Ras

![Figure 7](https://example.com-figure7.jpg)

**Figure 7.** Transfection of the hGH gene into NIH-3T3 cells stimulates proliferation and oncogenic transformation. A, foci formation in NIH-3T3 cells stably transfected with the hGH gene or a control vector as described. B, monolayer growth curve of NIH-3T3-vector and NIH-3T3-hGH cells. C, soft agar colony formation by NIH-3T3-vector and NIH-3T3-hGH cells. Columns, mean of triplicate determinations; bars, SD. *, *P < 0.01.

![Figure 8](https://example.com-figure8.jpg)

**Figure 8.** Autocrine hGH concomitantly activates a proliferative and antiapoptotic transcriptional program for anchorage-independent growth. RT-PCR (A) and Western blot analysis (B) of c-Myc, Cyclin D1, and Bcl-2 expression in MCF-10A-vector and MCF-10A-hGH cells. C, MCF-10A-vector and MCF-10A-hGH cells were transiently transfected with c-Myc, Cyclin D1, and Bcl-2 promoter reporter plasmids for determination of autocrine hGH–stimulated transcriptional activity. D, MCF-10A-vector and MCF-10A-hGH cells were transiently transfected with EphA2, c-Myc, Cyclin D1, and Bcl-2 promoter reporter plasmids in the presence or absence of **HOXA1** siRNA plasmid. E, quantification of soft agar colony formation by MCF-10A-hGH in the presence of control siRNA construct or siRNA construct for **HOXA1**, c-Myc, Cyclin D1, or Bcl-2. Columns, mean of triplicate determinations; bars, SD. *, *P < 0.01.
Autocrine hGH evidently also requires the presence of as yet undefined cofactors for oncogenic transformation, as colony formation in soft agar requires serum and in vivo tumor formation was only achieved in the presence of Matrigel (this study). We also have evidence that autocrine hGH increases telomerase activity in human mammary carcinoma,7 and this raises the intriguing possibility that enhanced expression of autocrine hGH may be sufficient to both immortalize and oncogenically transform the human mammary epithelial cell. However, the delineation between immortalization and oncogenic transformation as distinct processes may not be as clear as initially proposed (see ref. 33 for a review). This is evidenced by the observation that the overexpression of hTERT itself in the human mammary epithelial cell has been reported to cause the autocrine/paracrine secretion of multiple growth factors (34, 35) and, consequently, that simple hTERT overexpression by itself may contribute to tumor formation (36).

In any case, autocrine hGH has been established as an important promoter of oncogenic transformation.

We note here that the oncogenic transforming effect of hGH is exclusive to autocrine-produced hGH and is not observed with exogenous administration of hGH. The lack of effect of exogenous hGH on oncogenicity is concordant with a large database of patients (37) in whom administration of exogenous hGH does not alter the relative risk for development of mammary carcinoma. Microarray analysis of 19,000 human genes has identified a subset of 305 genes in a human mammary carcinoma cell line that are exclusively regulated by autocrine hGH8 although autocrine and exogenous hGH also regulated 167 common genes. It is therefore apparent that autocrine and exogenous hGH behave as distinct entities. Indeed, we have previously demonstrated that autocrine hGH, compared with exogenous hGH, differentially regulates the transcriptional activity of HOXA1, itself a powerful human mammary epithelial oncogene (19) and shown herein to be required for autocrine hGH–stimulated oncogenic transformation. Furthermore, we have also demonstrated that autocrine hGH differentially regulates apoptosis in comparison to exogenous hGH in human mammary carcinoma cells (16) related to differential gene transcription of antiapoptotic (CHOP/GADD153; ref. 18) and proapoptotic (PTGF-β; ref. 38) factors. What remains to be clarified is the precise mechanism for the differential oncogenic effect of autocrine hGH, although the subset of exclusively autocrine hGH regulated genes will presumably contain many of the oncogenic determinants of autocrine hGH. The differential mode of presentation of autocrine hGH to the cell (low concentration but continuous secretion as opposed to high but transient concentrations of exogenous hGH, discussed in ref. 39) may contribute to differential gene expression; analogously different secretory patterns of pituitary growth hormone possess disparate effects (40). In any case, we have demonstrated that autocrine hGH is capable of stimulating oncogenic transformation of human mammary epithelial cells, whereas exogenous hGH is not. The small but significant inhibition of autocrine hGH–stimulated oncogenic transformation by exogenous hGH may simply be due to shift of the bell-shaped hGH dose-response curve. hGH has been proposed to initiate signal transduction by homodimerization of its receptor, with high concentrations of conditions in which the cell is maintained in a nontransformed state. Autocrine hGH evidently also requires the presence of as yet undefined cofactors for oncogenic transformation, as colony formation in soft agar requires serum and in vivo tumor formation was only achieved in the presence of Matrigel (this study). We also have evidence that autocrine hGH increases telomerase activity in human mammary carcinoma,7 and this raises the intriguing possibility that enhanced expression of autocrine hGH may be sufficient to both immortalize and oncogenically transform the human mammary epithelial cell. However, the delineation between immortalization and oncogenic transformation as distinct processes may not be as clear as initially proposed (see ref. 33 for a review). This is evidenced by the observation that the overexpression of hTERT itself in the human mammary epithelial cell has been reported to cause the autocrine/paracrine secretion of multiple growth factors (34, 35) and, consequently, that simple hTERT overexpression by itself may contribute to tumor formation (36).

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6 S. Mukina and P.E. Lobe, unpublished observations.
7 Chen et al, submitted for publication.
8 Xu et al., in revision.
hGH inhibitory, due to excess binding of the higher affinity site to free receptor molecules (for a review, see ref. 26). Indeed, exogenous hGH does not significantly alter slow agar colony formation in MCF-7 vector cells in which no autocrine hGH is produced. Alternatively, exogenous hGH additional to autocrine hGH may interfere with receptor processing and/or availability or directly mitigate certain signal transduction processes stimulated by autocrine hGH.

Pathologic, environmental, nutritional, and pharmacologic factors that result in increased mammary epithelial hGH production will presumably contribute to neoplastic progression of the human mammary gland (12, 22, 41). In this regard, it is interesting that a known inhibitor of mammary carcinogenesis in vivo, vitamin D, also inhibits hGH gene transcription in a human mammary carcinoma cell line (42). Functional antagonism of hGH (12, 22) and the molecular pathways it uses (1, 2, 26), will therefore constitute novel adjunct therapeutic approaches to both the prevention and treatment of human mammary gland neoplasia.

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