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Growth Hormone Is Secreted by Normal Breast Epithelium upon Progesterone Stimulation and Increases Proliferation of Stem/Progenitor Cells

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SUMMARY

Using in vitro and in vivo experimental systems and in situ analysis, we show that growth hormone (GH) is secreted locally by normal human mammary epithelial cells upon progesterone stimulation. GH increases proliferation of a subset of cells that express growth hormone receptor (GHR) and have functional properties of stem and early progenitor cells. In 72% of ductal carcinoma in situ lesions, an expansion of the cell population that expresses GHR was observed, suggesting that GH signaling may contribute to breast cancer development.

INTRODUCTION

Mammary gland development is controlled by the endocrine system, in particular by the ovarian steroid hormones, estrogen and progesterone, and by the pituitary hormones, growth hormone (GH) and prolactin. Studies in animal models showed that GH deficiency impairs mammary gland development. Spontaneous dwarf rats, which bear a loss-of-function mutation in GH, have deficient alveolar development that can be rescued by GH reinfusion (Swanson and Unterman, 2002). *Ghr* knockout (KO) mice have retarded duct development and limited side branching (Bocchinfuso and Korach, 1997; Zhou et al., 1997). In humans, mutations affecting the expression and function of the GH receptor (GHR) are collectively known as Laron syndrome (LS). Similar to *Ghr* KO mice, these patients have short stature and reduced body weight (Laron and Klinger, 1994). Mammary gland development is affected but can support normal lactation.

Sustained exposure to steroid hormones constitutes one of the best established factors of risk for breast cancer (Russo and Russo, 2006). There is compelling evidence, from both animal work and epidemiological studies, that elevated levels of GH also increase the risk of breast cancer (De Stavola et al., 2004; Gunnell et al., 2001). The incidence of cancers is higher in patients with acromegaly, a condition associated with hypersecretion of GH (Jenkins, 2004; Perry et al., 2008; van Garderen and Schalken, 2002; Waters and Barclay, 2007), and in individuals with taller height (Ahlgren et al., 2004; Green et al., 2011; De Stavola et al., 2004; Gunnell et al., 2001). Conversely, no cancers have been diagnosed so far in patients with LS (two cohorts studied, of 169 and 230 patients), although they have a higher longevity than the general population (Laron, 2008). Their blood relatives had an incidence of cancers of 24%.

There is evidence that GH can be secreted by breast cancer cells (Chiesa et al., 2011; Raccurt et al., 2002). Studies from Lobie's group have reported that autocrine GH signaling in MCF7 cells confers a mesenchymal, invasive phenotype in vitro and generates more aggressive tumors in vivo (Mukhina et al., 2004). Although the molecular mechanisms underlying steroid hormones and GH signaling have been elucidated in studies spanning decades of research, it is still poorly understood how exposure to these hormones increases risk of breast cancer.

In this study, we utilized a combination of in vitro and in vivo functional assays and in situ analysis of normal breast epithelium to show that GH selectively exerts its effects on normal mammary stem/progenitor cells. We demonstrated that GHR is expressed in a distinct subpopulation of cells with phenotypic and functional properties of stem and early progenitor cells. We also showed that a subpopulation of breast epithelial cells produces GH upon progestin stimulation. GH/GHR signaling increases proliferation of mammary stem and progenitor cells. We speculate that sustained GH stimulation, linked to sustained progesterone stimulation, can increase the risk of malignant transformation by expanding the stem/progenitor cell population and increasing their proliferation rate. Consistent with this concept, we found that 90% of ductal carcinoma in situ (DCIS) lesions have a GHR+ cell population detectable by immunohistochemistry (IHC). In 72%

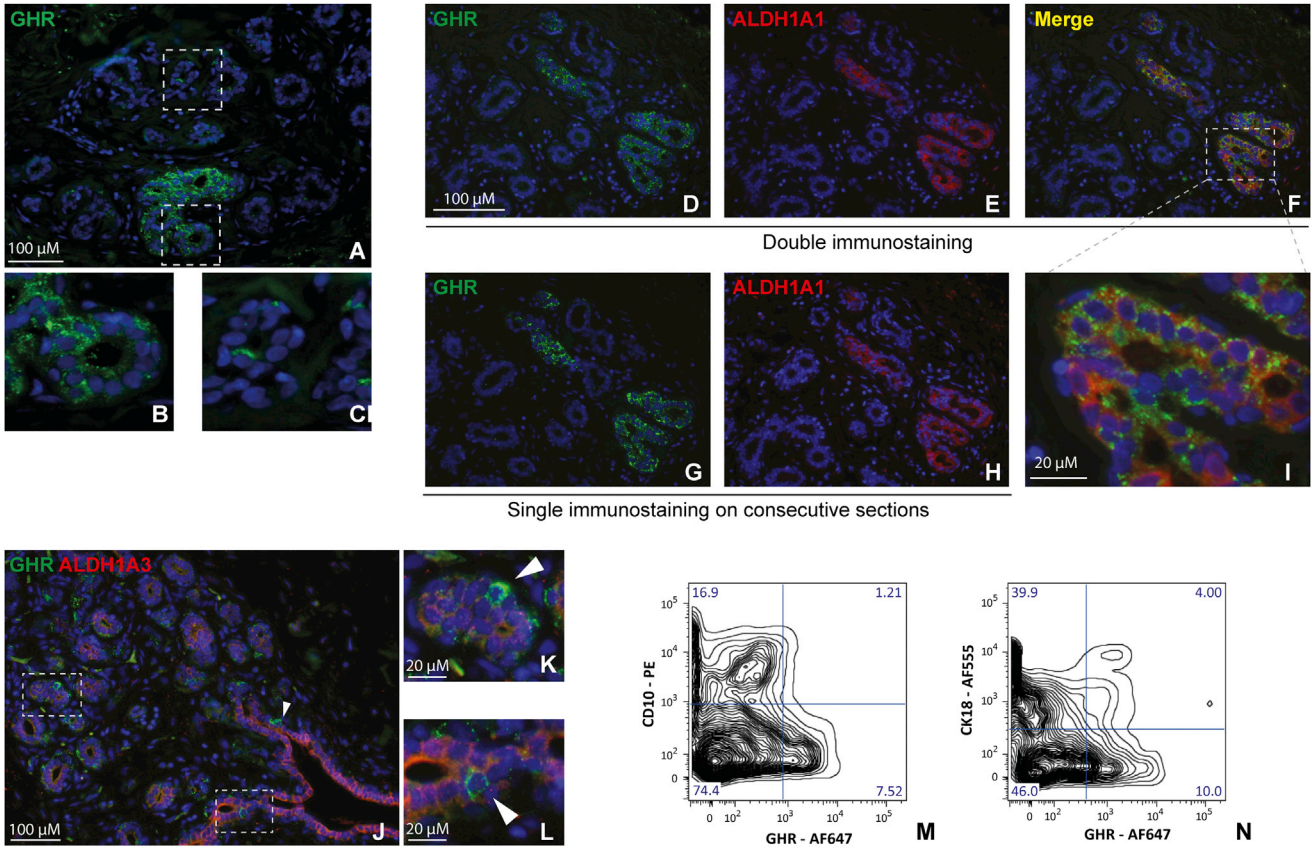

Figure 1. GHR Is Expressed in the Human Mammary Gland Epithelium

(A–C) Immunostaining of normal breast epithelium shows that GHR is expressed mostly in clusters and rarely as scattered cells. Representative images of eight samples.

(D–F) Double IF of normal breast epithelium shows that GHR is expressed mostly in ALDH1A1+ cells or in their immediate proximity.

(G and H) Single IF for GHR and ALDH1A1 in consecutive normal breast sections.

(I) Detail of (E), showing ALDH1A1+/GHR+ cells.

(J–L) Double IF for ALDH1A3 and GHR shows isolated GHR+ cells adjacent to ALDH1A3+ cells (arrowheads). Representative images of five samples.

(M) Flow cytometry for GHR and CD10 showing no overlap between cells expressing these markers. Representative data from two different samples.

(N) Flow cytometry for GHR and CK18 showing a small overlap (4%) between these markers. Representative data from two different samples.

of DCIS, the GHR+ cell population is expanded compared to normal tissue. We also showed that inhibition of GH signaling halts the growth of a patient-derived breast cancer xenografted in immunodeficient mice.

RESULTS

GHR Is Present in a Subset of Normal Human Breast Epithelium Cells that Express Stem Cell Markers and Lack Lineage Differentiation Markers

GHR Is Expressed in the Normal Human Mammary Epithelium

We performed immunofluorescent (IF) staining for GHR on normal human breast sections (aesthetic mammoplasty samples). GHR was detected in all samples analyzed, originating from eight patients. The vast majority of GHR+ cells in the epithelium were present in cell clusters, and a small minority were present as scattered, isolated cells (Figures 1A–1C). GHR+ cells were present in 1.2%–5% of mammary epithelial cells (four patients, three paraffin blocks/ sample, 4,359 ± 2,555 average number cells analyzed/sample). We utilized flow cytometry analysis for a more sensitive and quantitative assessment and found that GHR was expressed in 3.5%–19% of normal breast epithelial cells (mean = 9.7 ± 6.27 SD, n = 6) (staining controls are shown in Figures S1A–S1F available online).