Expression of GHRH-R, a Potentially Targetable Biomarker, in Triple-negative Breast Cancer

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Purpose: Growth hormone–releasing hormone (GHRH) has been shown to modify the growth behavior of many cancers, including breast. GHRH is produced by tumor cells, acts in an autocrine/paracrine manner, and requires the presence of GHRH receptor (GHRH-R) on the tumor cells to exert its effects. GHRH activity can be effectively blocked by synthetic antagonists of its receptor and hence, the expression of GHRH-R by tumor cells could serve as a predictor of response to GHRH-R antagonist therapy. In this study, we investigated the expression of GHRH-R in triple-negative breast cancers (TNBC). As TNBCs are morphologically and immunophenotypically heterogenous, the staining results were also correlated with the histologic subtypes of these tumors.

Materials and Methods: On the basis of histomorphology and immunophenotype, 134 cases of primary TNBCs were further subdivided into medullary, metaplastic, apocrine, and invasive ductal carcinomas of no special type (IDC-NST). Immunohistochemistry for GHRH-R was performed on paraffin sections and the staining results were assessed semiquantitatively as negative, low expression, moderate, and high expression.

Results: Of the 134 TNBCs, 85 were classified as IDC-NST, 25 as metaplastic, 16 as medullary, and 8 as apocrine carcinoma. Overall, positive reaction for GHRH-R was seen in 77 (57%) of tumors including 66 (77.6%) of IDC-NST. All medullary carcinomas were negative for GHRH-R and, with the exception of 1 case with low expression, none of the metaplastic carcinomas expressed GHRH-R ($P < 0.005$).

Conclusions: A considerable number of TNBCs are positive for GHRH-R as a predictor of potential response to anti-GHRH-R treatment. This expression however, varies considerably between histologic subtypes of triple-negative breast cancers. Although most medullary and metaplastic carcinomas do not express GHRH-R, three fourths of the IDC-NST show a positive reaction. Testing for GHRH-R expression is therefore advisable if anti-GHRH-R therapy is being considered.

Key Words: triple-negative breast cancer, GHRH, GHRH-R, GHRH-R antagonists, histologic types of triple-negative breast cancer

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Triple-negative breast cancer (TNBC) are a heterogenous group of breast cancers that lack the expression of estrogen receptor (ER), progesterone receptor (PR), and HER2. As a group they have a more aggressive clinical behavior with a higher risk of local and distant recurrence. Surgery and chemotherapy remain the mainstay of treatment, although a number of new targeted drugs have shown promising results. As antagonists of growth hormone–releasing hormone receptor (GHRH-R) have been shown to suppress the growth of mammary cancer, we evaluated the expression of this biomolecular marker as a potential therapeutic target in a cohort of TNBCs. Also, because these neoplasms are morphologically and immunophenotypically diverse, the GHRH-R expression results were correlated with the histologic subtypes of each tumor.

MATERIALS AND METHODS

Excisional biopsies from 134 consecutive cases of untreated triple-negative breast carcinomas (TNBC) from the files of the Department of Pathology, University of Miami/Jackson Memorial Medical Center and Sylvester Cancer Center were evaluated for the immunohistochemical expression of GHRH-R. All specimens had been previously analyzed for ER, PR, and HER2.

The diagnosis of triple-negative mammary carcinoma was confirmed in all cases. In those cases that based on histology a medullary, metaplastic or apocrine carcinoma was suspected, the following immunohistochemical markers were used for confirmation: HLA-DR for medullary, P63 for metaplastic, androgen receptor, and GCDFP-15 for apocrine carcinoma.

Formalin-fixed, paraffin-embedded 4 μm sections were used for immunohistochemistry, following heat-induced antigen retrieval and a polymer detection system, in the Bond III Autostainer (Leica Microsystem, Buffalo Grove, IL). Diaminobenzidine was used as the chromogen

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TABLE 1. Antibody Clones, Working Dilutions, and the Commercial Sources

Antibody	Clone	Working Dilution	Commercial Source
GHRH-R	Ab28692	1:50	AbCam
HLA-DR	LN3	1:400	Biogenex
P63	4A4	Ready to use	BioCare
Androgen receptor	SP107	Ready to use	Cell Marque
GCDFP-15	23A3	Ready to use	Leica

GHRH indicates growth hormone–releasing hormone; GHRH-R, GHRH receptor.

in the presence of hydrogen peroxide. The cytoplasmic and cytoplasmic membrane staining of normal anterior pituitary was used as positive control, whereas replacement of the primary antibody with nonimmune mouse or rabbit serum served as the negative control. The clone, commercial source and the working dilution of each antibody is depicted in Table 1. On the basis of the percentage of positive cells and the intensity of reaction, the immunohistochemical reaction for GHRH-R was calculated by the H-Score. The semiquantitative scores ranged from 0 (no staining), to 1+ (low expression), 2+ (moderate expression), and 3+ (high expression). For comparison, an additional 50 examples of primary mammary carcinomas that were hormone-positive and/or HER2-positive were similarly stained for GHRH-R.

RESULTS

Of the 134 cases of TNBC, 25 (18%) were classified as metaplastic, 16 (12%) as medullary, 8 (6%) as apocrine, and 85 (63%) as infiltrating ductal carcinomas of no special type (IDC-NST).

Overall, positive expression of GHRH-R was seen in the cytoplasmic membrane and/or cytoplasm of 77 (57%) of triple-negative tumors (Table 2). Of the 85 IDC-NST, 66 (76.7%) displayed the expression of GHRH-R, 37 or 56% of which showed moderate to high expression (Fig. 1). Six (75%) of apocrine carcinomas showed moderate to high GHRH-R expression, whereas all medullary carcinomas were negative for GHRH-R (Fig. 1) and, with the exception of 1 case with low expression, none of the metaplastic carcinomas expressed GHRH-R ($P < 0.005$). Positive GHRH-R expression was seen in 36 (72%) of hormone and/or HER2-positive tumors ($P = 0.36$ vs. IDC-NST).

DISCUSSION

Triple-negative mammary carcinomas do not express ER, PR, or HER2. They account for ~12% to 15% of breast cancers with distinctive clinical features and aggressive behavior.[1–4] TNBCs occur more commonly in younger women and in those with *BRCA*-1 gene abnormality.[5] As a group, TNBCs have a higher risk of local recurrence and distant metastasis.[6] At the present time surgery and cytotoxic chemotherapy remains the mainstay of management for these patients. Although no current standard treatment modality is universally agreed upon, a number of targeted therapeutic approaches have shown promising results.[7] These include various poly ADP ribose polymerase inhibitors,[8] androgen receptor antagonists[9,10] glucocorticoid receptor antagonists,[11] and immune checkpoint inhibitors such as anti-PD-1/PD-L1.[12]

It is known however, that TNBCs are a diverse group of tumors with different histomorphology, immunophenotype, molecular fingerprints, and most importantly, clinical behavior.[13–16] They range from relatively indolent types, such as salivary gland-like tumors,[17,18] to less aggressive medullary types, to highly malignant metaplastic carcinomas.[19–22] It has also been shown that histologic subtypes of TNBC vary considerably in the expression of biomolecular targets of therapy.[23] Consequently, a "one-size-fits-all" approach to targeted therapy may lead to disappointing results in various clinical trials. In the current study the expression of GHRH-R, a potentially targetable biomarker, was assessed in relation to immunohistochemically confirmed histological types of TNBCs.

GHRH is secreted by the hypothalamus and regulates the release of growth hormone from the anterior pituitary gland.[24] In addition to this putative and nominal endocrine role, it has been shown that GHRH acts as a growth factor in a diverse group of malignancies. It requires, however, the presence of its receptor, GHRH-R, to exert its effect on neoplastic cells.[24,25] GHRH-R and its biologically active splice variant, SV-1, are present in a number of neoplastic tissues; they may also exhibit their activity independently of ligand binding.[25,26] Antagonists of GHRH-R have been demonstrated experimentally to inhibit the growth of a number of human tumors in vitro, including various types of breast cancer.[27–32] Furthermore, the presence of GHRH and GHRH receptors have also been reported in human breast cancer cell lines, suggesting that in some human breast cancers, local GHRH and its receptor could form an autocrine

TABLE 2. GHRH-R Expression is Histologic Types of Triple-negative Breast Cancers

Histologic Type	Number	GHRH-R Negative	GHRH-R Positive Low	GHRH-R Positive Moderate/Strong
Medullary carcinoma	16	16	0	0
Metaplastic carcinoma	25	24	1	0
Apocrine carcinoma	8	2	0	6
IDC-NST	85	19	29	37
Total	134	61	30	43

IDC-NST indicates infiltrating ductal carcinoma of no special type; GHRH, growth hormone–releasing hormone; GHRH-R, GHRH receptor.

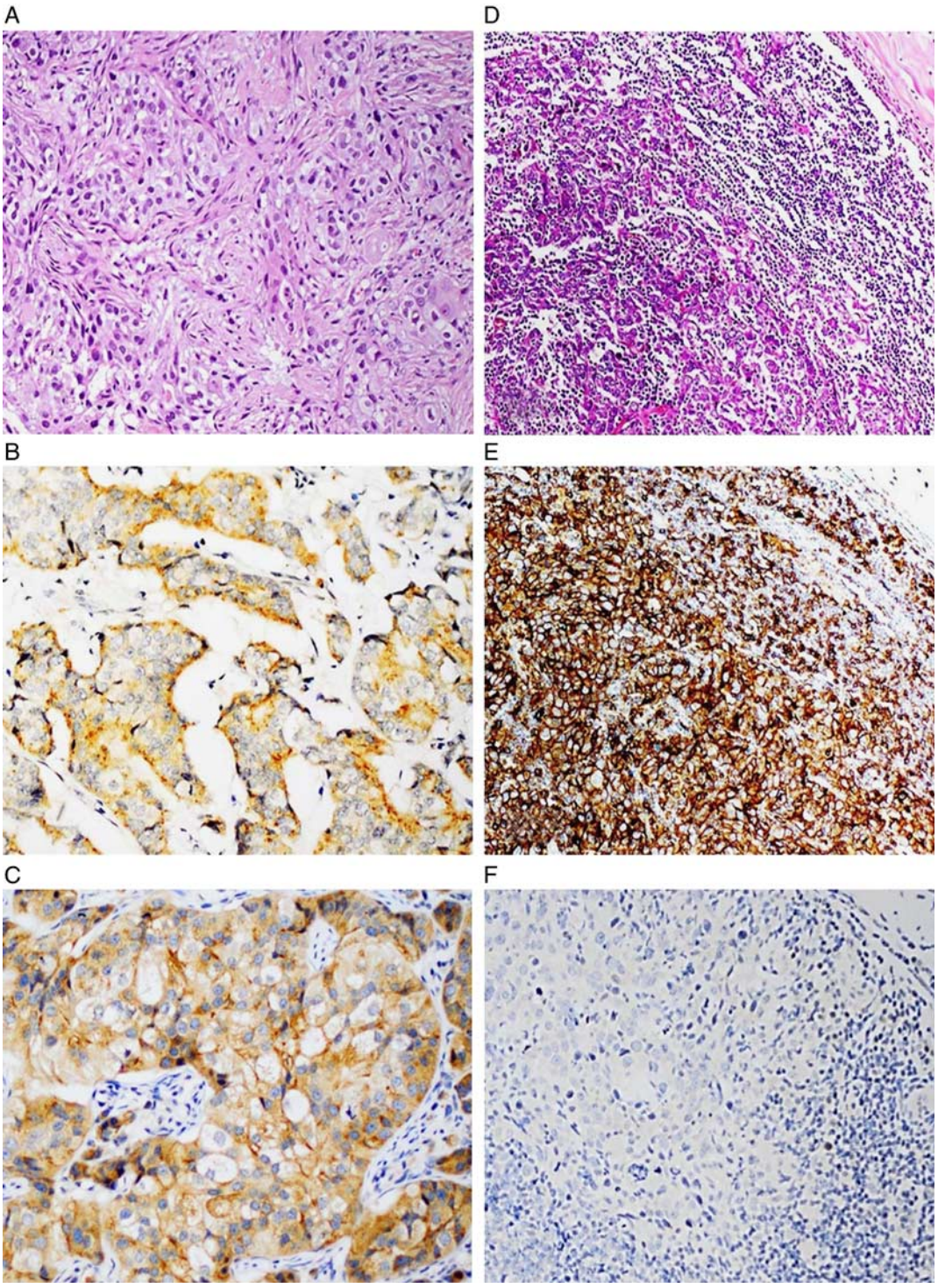

FIGURE 1. A, Infiltrating ductal carcinoma, of no special type, hematoxylin and eosin, ×50. B, Predominant cytoplasmic membrane staining reaction of GHRH-R, ×100. C, Cytoplasmic and cytoplasmic membrane reaction of GHRH-R in a high-grade triple-negative breast carcinoma, ×100. GHRH indicates growth hormone–releasing hormone; GHRH-R, GHRH receptor. D, Typical medullary carcinoma of the breast, hematoxylin and eosin, ×50. E, Tumor cells and lymphocytes are positive for HLA-DR, ×50. F, Tumor cells are negative for GHRH-R, ×50. GHRH indicates growth hormone–releasing hormone; GHRH-R, GHRH receptor.

mitogenic loop that may participate in controlling the growth of malignant cells.[26,32,33] The existence of such an autocrine loop has also been suggested in TNBCs.[34] In vitro studies have demonstrated that targeting the GHRH receptors by peptide antagonists alone, or in combination with conventional chemotherapy, effectively inhibits the growth of TNBC cell lines.[31,34,35] In vivo treatment of nude mice bearing TNBC xenographs with GHRH antagonists has also been demonstrated to suppress the growth of these tumors.[31,35]

To our knowledge, this is the first study that has demonstrated the expression of GHRH-R in clinical samples of TNBCs. Furthermore, we have shown that the expression of GHRH-R in these tumors varies considerably and it very much depends on the histologic types of TNBC. It has been established that on the basis of gene expression profiles, a number of TNBCs show a basal-like genotype.[36,37] Traditionally, basal/myoepithelial cytokeratins have been advocated as surrogate immunohistochemical markers for these basal-like genotypes.[38–40] More recent studies however, have questioned the use of molecular profiling as the "gold standard" in general, and the basal cytokeratin immunohistochemistry for basal-like genotypes, in particular.[41–43] Therefore, in this study, we chose not to use the controversial term "basal-like" and instead, concentrated on morphologically and immunophenotypically distinct types of TNBCs, as they could be objectively characterized by their histologic and immunohistochemical features. For example, although metaplastic mammary carcinomas show a wide spectrum of histomorphologic subtypes, from the classic squamous cell carcinomas to matrix-forming carcinomas, spindle cell carcinomas, and carcinosarcomas,[44–46] they share the same immunophenotype by expressing p63.[47,48] With the exception of 1 case with weak staining, none of the metaplastic carcinomas in our study expressed GHRH-R. Similarly, it has been shown that most typical medullary carcinomas express HLA-DR, a component of the MHC class II antigenic system.[49–51] All of our examples of typical medullary carcinoma were negative for GHRH-R.

The majority of apocrine mammary carcinomas are triple-negative.[52] In addition to their distinctive histomorphology, all apocrine carcinomas express nuclear androgen receptor and cytoplasmic GCDFP-15.[53] In this series 75% of apocrine carcinomas expressed GHRH-R. This study also shows that the rate of GHRH-R expression in triple-negative ductal carcinoma of no special type is comparable with that of hormone and/or HER2-positive tumors.

In summary, the expression of GHRH-R varies considerably among the various histologic types of TNBC. Most notably, metaplastic and medullary carcinomas are negative for GHRH-R, whereas positive reactions are seen in about 77% of other types. It is imperative therefore, to test patients with TNBCs for the expression of GHRH-R to correctly establish their possible candidacy for effective targeted treatment by GHRH-R antagonists.

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