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Co-expression of GPR30 and ER β and their association with disease progression in uterine carcinosarcoma

Gloria S. Huang, MD^{1,2,3}, Marc J. Gunter, PhD⁴, Rebecca C. Arend, MD¹, Maomi Li, MD, PhD⁵, Hugo Arias-Pulido, PhD⁶, Eric R. Prossnitz, PhD⁸, Gary L. Goldberg, MD^{1,3}, and Harriet O. Smith, MD^{1,3}

¹Department of Obstetrics & Gynecology and Women's Health, Division of Gynecologic Oncology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York 10461.

²Department of Molecular Pharmacology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York 10461.

³Albert Einstein Cancer Center, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York 10461.

⁴Department of Epidemiology and Population Health, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York 10461.

⁵Department of Pathology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York 10461.

⁶Translational Therapeutics Laboratory, The University of New Mexico Cancer Center, Albuquerque, New Mexico 87131.

⁷Department of Cell Biology and Physiology, The University of New Mexico Cancer Center, Albuquerque, New Mexico 87131.

Abstract

Objective—To evaluate the expression of G-coupled protein receptor 30 (GPR30) and estrogen receptor beta (ER β) in uterine carcinosarcoma.

Study Design—Immunohistochemistry was performed using antibodies to GPR30, ER β , estrogen receptor alpha (ER α), and progesterone receptor (PR). The staining intensity and percentage of positive cells were scored for each tissue section. Expression levels were compared using the Wilcoxon rank-sum test. Correlation was evaluated by Spearman's rho and logistic regression.

Results—Compared with normal endometrium, carcinosarcoma had lower ER α and PR expression (both $p < 0.01$) but higher GPR30 epithelial expression ($p = 0.03$). Advanced stage carcinosarcoma had higher GPR30 ($p < 0.01$) and ER β ($p = 0.02$) epithelial expression compared with early stage carcinosarcoma. Expression of GPR30 and ER β correlated with each other ($p < 0.01$), and not with ER α or PR.

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Corresponding Author: Gloria S. Huang, M.D., Division of Gynecologic Oncology, 1695 Eastchester Road, Suite 601, Bronx, New York 10461, Telephone (718) 405-8082, Facsimile (718) 405-8087, gloria.huang@einstein.yu.edu.

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Conclusions—In uterine carcinosarcoma, GPR30 and ER β are coordinately overexpressed and expression levels increase in advanced stage disease, supporting the involvement of alternative estrogen receptors in disease progression.

Keywords

GPR30; Estrogen receptor; uterine carcinosarcoma

Introduction

Carcinosarcoma of the uterus is a highly aggressive tumor composed of mixed malignant epithelial and mesenchymal components.¹ Although these tumors are relatively uncommon, accounting for only 4–9% of all uterine cancers, they are associated with disproportionately higher mortality rates compared with other corpus malignancies.² While the etiopathogenesis of carcinosarcomas remains poorly understood, these tumors share epidemiologic risk factors with endometrioid-type endometrial carcinomas, including obesity and exposure to selective estrogen receptor modulators (SERMs).^{3–5} These observations support a potential role of dysregulated estrogen signaling in the development of this tumor.

Estrogen is a central regulator of growth, differentiation, and function of reproductive tissues, including the uterus. The classical estrogen receptors are comprised of the related nuclear hormone receptors estrogen receptor alpha (ER α) and estrogen receptor beta (ER β), which are co-expressed in the ovary and uterus during fetal development.⁶ In the adult reproductive tract, ER β expression decreases relative to ER α in the uterus, while both isoforms continue to be expressed in the ovary. The downstream effects of estrogen receptor subtype signaling appear to be highly tissue specific, with opposing actions in different organs. For example, in the ovary, loss of ER β expression has been implicated in the development of ovarian cancer,^{6–8} and in the prostate and breast, ER β appears to oppose the proliferative activity of ER α .⁹ Conversely, ER β overexpression has been implicated in the pathogenesis of endometriosis, a disease characterized by ectopic proliferation of endometrial tissue.¹⁰ In this disease, ER β was found to be highly overexpressed in endometriotic stromal cells compared with normal endometrium. Furthermore, in ovarian endometriosis, the overexpression of ER β was shown to disrupt autoregulatory feedback, resulting in increased local levels of estradiol and pro-inflammatory factors.¹¹

We have previously reported our novel findings regarding the prognostic value of ER subtype expression in uterine carcinosarcoma. ER α expression was associated with an approximate 70% reduced risk for death in univariate survival analysis.⁵ In contrast, ER β expression was significantly elevated in uterine carcinosarcoma, compared with normal endometrium, and high ER β expression was significantly associated with advanced stage disease.¹²

A novel intracellular 7-transmembrane G protein-coupled receptor (GPR30), also known as the G protein-coupled estrogen receptor (GPER), has been identified and characterized by our group and others.^{13–15} GPR30 has been shown to mediate cellular responses to estrogen, independent of ER α and ER β . Of note, GPR30 is stimulated by not only estrogen, but also by tamoxifen (a prototypical SERM). Ligand stimulation of GPR30 results in cyclic AMP production and calcium mobilization as well as c-Src, MAP kinase and phosphatidylinositol 3-kinase activation, mediated by epidermal growth factor (EGF) receptor transactivation.¹⁶

To examine the role of GPR30 in endometrial cancer, our group performed a cross-sectional analysis of GPR30 tumor expression in 47 patients with diverse histological types of endometrial cancer.¹⁷ The findings demonstrated that GPR30 was overexpressed more frequently in high-risk tumors, including three carcinosarcomas evaluated in the study. In

addition, GPR30 expression was significantly associated with clinical and pathological predictors of poor survival in endometrial cancer, such as surgical stage and myometrial invasion. Similarly, in a recent analysis of primary ovarian tumors, GPR30 expression was significantly associated with higher tumor grade and advanced stage, while in breast cancer, high GPR30 expression has been correlated with the presence of distant metastases.^{18, 19}

GPR30 expression was not assessed in our prior carcinosarcoma studies,^{5, 12} nor have data on GPR30 expression in this tumor type been reported in the literature. Given our observation of ER β overexpression in carcinosarcoma, particularly those of advanced stage, and the association of GPR30 expression with biologically aggressive endometrial neoplasia, we hypothesized that these alternative estrogen receptors may be coordinately expressed in uterine carcinosarcoma, in association with disease progression. Therefore, the aim of the current study was to evaluate the expression of GPR30 in uterine carcinosarcomas and its relationship with ER isoform expression and clinical/pathological factors.

Materials and Methods

This study was approved by the Montefiore Office of Research and Sponsored Programs and Office of Institutional Board Review and was classified as exempt per federal regulations 45 CFR46.101(b). Patients treated for uterine carcinosarcoma at Montefiore Medical Center between 1995 and 2003 were identified and clinicopathologic data were abstracted from medical records. As previously described, a de-identified tissue microarray (TMA) containing uterine carcinosarcomas (N=24) and normal endometrium (N=8) was constructed using triplicate to quadruplicate cores of representative areas of tumor from early (FIGO stage I/II) and advanced (FIGO stage III/IV) patients, including both primary and, where available, metastatic sites.¹² Normal endometrial tissue was obtained from hysterectomy specimens that did not contain evidence of benign or malignant endometrial pathology.

Immunohistochemistry

GPR30 immunohistochemistry was performed using a rabbit polyclonal affinity-purified antibody directed against the C-terminus of GPR30, using previously described methodology.¹⁷ The methodology for immunohistochemistry using antibodies to ER α (Clone 1D5; Dako North America, Carpinteria, CA); ER β (Clone 14C8; GeneTex, San Antonio, TX); and PR (Clone PgR 636; Dako North America) were described in a prior manuscript.¹²

Interpretation of immunohistochemical staining was performed by two investigators, a pathologist (M.L.) and a gynecologic oncologist (H.O.S.). For each core, the staining intensity (0, 1+, 2+, 3+) and the percentage of cells staining positive (0–100%) were determined separately for the epithelial and stromal components. An H-score was calculated as the product of the intensity and the percentage of cells with positive staining. The individuals involved in staining and grading were blinded to the clinical information until after completion of these steps.

Statistical Analysis

The categorical data were summarized by computing frequency distributions for each group, and differences in variable distributions were evaluated using the χ^2 statistic. Standard descriptive statistics were used to summarize H-scores and continuous variables. The association of clinicopathologic variables with stage was evaluated using χ^2 analysis and Fisher exact methods, as appropriate. Comparison of receptor expression levels between carcinosarcoma and normal endometrium, and between early and advanced stage CS, was made using the Wilcoxon rank sum test. Correlations between receptor expression levels were evaluated with Spearman's rank correlation coefficient.

Results

Clinicopathologic variables

Early stage (stage I/II) and advanced stage (stage III/IV) carcinosarcoma cases were classified according to the FIGO surgical staging system for uterine corpus cancer. The early and advanced stage patients were not significantly different with respect to age, body mass index (BMI), and racial distribution (Table 1). Among patients with advanced stage disease, serous or clear cell histology of the epithelial component was observed at a higher frequency compared with the frequency observed in early stage patients, although this was not statistically significant (62% vs. 18%; $p=0.12$). Deep myometrial invasion and lymphovascular invasion occurred frequently in both groups. Among patients with advanced stage disease, 46% had adnexal metastases and 56% had nodal metastases. The use of adjuvant radiation and chemotherapy was common and did not significantly differ between early and advanced stage patients.

Classical hormone receptor expression

As shown in Figure 1, carcinosarcoma exhibited decreased ER α and PR expression in the glandular and stromal compartments, compared with normal endometrium (all $p<0.01$). Similar low expression of ER α and PR was observed in both early stage and advanced stage carcinosarcoma, as previously reported.¹² ER α and PR expression were positively correlated with each other in both the glandular and stromal components of carcinosarcoma; Spearman correlation coefficients for ER α and PR were 0.69 and 0.58, in the glandular and stromal compartments, respectively (both $p<0.01$).

GPR30 and ER β expression

As shown in Figure 2, GPR30 expression was elevated in the glandular component of carcinosarcoma compared with normal endometrial epithelium ($p=0.03$). In addition, advanced stage carcinosarcoma demonstrated significantly higher GPR30 glandular expression compared with early stage carcinosarcoma ($p<0.01$). GPR30 stromal expression was not significantly altered compared to normal endometrial epithelium. ER β glandular expression was elevated in advanced stage compared with early stage carcinosarcoma ($p=0.02$).

The relationship of ER subtype expression and GPR30 expression was evaluated. ER β glandular and stromal expression were positively correlated with each other ($r=0.59$, $p=0.02$), and both were positively correlated with GPR30 stromal expression in carcinosarcoma ($r=0.66$ and $r=0.59$ respectively, both $p<0.01$). In contrast, ER α and GPR30 expression were not significantly correlated in normal endometrium. GPR30 and ER β expression were not correlated with ER α or PR expression.

Comment

In uterine carcinosarcoma, GPR30 and ER β expression levels are elevated compared with normal endometrium, and higher expression levels are observed in advanced stage disease compared with early stage disease. The expression of GPR30 and ER β in these malignant tumors appears to be independent of the classical hormone receptors, ER α and PR, both of which are both suppressed in uterine carcinosarcoma. These data support a clinically relevant role for these alternate estrogen receptors in carcinogenesis and disease progression in uterine carcinosarcoma.

We also observed a significant correlation between GPR30 and ER β expression in the glandular and stromal components of the carcinosarcomas, suggestive of coordinate regulation or crosstalk between these receptors in this disease. Supporting the existence of coordinate

regulation, it was reported that estradiol treatment of endometrial cells leads to an increase in GPR30 mRNA levels and that ER β directly regulates GPR30 levels at the mRNA level.²⁰ Alternatively, ER β may indirectly regulate the expression of GPR30 via downstream intracellular signaling events. For example, ER β -selective agonists have been shown to activate MAP kinase signaling in non-small cell lung cancer cells,²¹ and activation of the EGFR-MAP kinase pathway is known to upregulate GPR30 mRNA and protein levels in breast cancer cells.^{22, 23} It is also plausible that both GPR30 and ER β are coordinately upregulated due to the underlying genomic alterations that have been observed in carcinosarcoma, such as amplification of c-myc and ZNF217, observed in 78% and 87% of these tumors.²⁴ Further investigation is warranted to determine the mechanism by which GPR30 and ER β are upregulated in uterine carcinosarcoma.

There have been few studies of GPR30 expression in primary human tumors. To our knowledge, the present study is the first to evaluate GPR30 expression in carcinosarcomas. Consistent with the previously reported studies of GPR30 expression in ovarian,¹⁹ endometrial,¹⁷ and breast cancer,¹⁸ high GPR30 expression was found to be significantly correlated with advanced stage disease in this study of carcinosarcomas. The association of GPR30 and disease progression suggests a potential role of GPR30 signaling in motility and invasion, characteristic features of cells with metastatic potential. Indeed, a recently published study found that hydroxytamoxifen (OHT) stimulated the migration of ER-negative breast cancer cells, as analyzed by the Boyden chamber migration assay.²⁵ The ability of OHT to stimulate migration in these cells was dependent on GPR30 signaling, suggesting that GPR30 could mediate estrogen-driven metastases in the absence of classical estrogen receptors. In the present study, GPR30 elevation was predominantly observed in the glandular component of carcinosarcoma, and it is the malignant glandular cells that comprise the vast majority of metastatic lesions in this disease. The ability of GPR30 knockdown by RNA interference to abrogate cellular migration supports the validity of targeting this pathway therapeutically.²⁵ The recent identification of a GPR30-specific antagonist will be invaluable for ongoing investigations and potential translation of these findings into the clinical arena. 26

In summary, we have identified high expression of the estrogen receptors GPR30 and ER β to be significantly associated with disease progression in uterine carcinosarcoma. These results support undertaking further investigation and validation of these hormone receptor alterations in an additional, independent group of carcinosarcoma patients. Given the rarity of this disease, a multi-institutional collaboration is planned to increase the sample size and to evaluate the effect of GPR30 and ER β expression on recurrence and survival. In future studies, it will also be of interest to elucidate the upstream regulators and downstream effectors of receptor activation, and to examine the potential crosstalk between these receptors. The novel findings of this exploratory study, the first to evaluate GPR30 expression in carcinosarcoma, indicate that alternate estrogen signaling pathways represent a potential therapeutic target for this highly aggressive tumor type.

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Figure 1. Classical hormone receptor expression in benign endometrium and uterine carcinosarcoma

Shown in Panel A are representative tissue sections of benign endometrium (Normal) and carcinosarcoma (CS); immunohistochemical staining was performed with antibodies to ER β and PR.

Shown in Panel B are the H-scores (mean $\pm$ SD) for the glandular and stromal compartments of normal (NL) and carcinosarcoma (CS) tissue samples for ER α expression (blue bars) and PR expression (red bars).

Figure 2. GPR30 and ERβ expression in early and advanced stage uterine carcinosarcoma

Shown in Panel A are representative tissue sections of the primary tumors from a patients with Stage I uterine carcinosarcoma and Stage III uterine carcinosarcoma; also shown is a tissue section of a metastatic site from a patient with Stage III uterine carcinosarcoma. Immunohistochemical staining was performed with antibodies to GPR30 and ERβ.

Panel B (left) depicts the H-scores (mean ± SD) for GPR30 expression (green bars) and ERβ expression (purple bars) in the glandular and stromal compartments of normal (NL) and carcinosarcoma (CS) tissue samples. The H-scores (mean ± SD) for GPR30 expression (green bars) and ERβ expression (purple bars) for the glandular and stromal compartments of early stage (Stage I/II) and advanced stage (III/IV) carcinosarcoma are also shown in Panel B (right).

TABLE 1

Clinicopathological variables

	Stage I/II		Stage III/IV		P-value
Age	N	%	N	%	
Below 68	7 / 11	64	4 / 13	31	0.22
68 or older	4 / 11	36	9 / 13	69	
BMI					
Below 30	7 / 11	64	11 / 13	85	0.36
30 or higher	4 / 11	36	2 / 13	15	
Ethnicity					
Caucasian	5 / 11	45	5 / 13	38	0.41
African-American	2 / 11	18	6 / 13	46	
Hispanic	2 / 11	18	2 / 13	15	
Asian-American	1 / 11	9	0 / 13	0	
Missing	1 / 11	9	0 / 13	0	
Carcinoma component					
Endometriod/NOS	8 / 11	73	5 / 13	38	0.12
Serous/Clear cell	2 / 11	18	8 / 13	62	
Missing	1 / 11	9	0 / 13	0	
Myometrial Invasion					
None/Less than Half	2 / 11	18	0 / 13	0	0.20
More than half	9 / 11	82	13 / 13	100	
Lymphovascular Invasion					
Yes	6 / 11	55	12 / 13	92	0.06
No	5 / 11	45	1 / 13	8	
Adnexal Involvement					
Yes	0 / 11	0	6 / 13	46	0.02
No	11 / 11	100	7 / 13	54	
Node Status					
Positive	0 / 11	0	5 / 13	38	<0.01
Negative	11 / 11	100	4 / 13	31	

	Stage I/II		Stage III/IV		P-value
	N	%	N	%	
Missing	0	11	4	13	31
Adjuvant Radiation					
Yes	9	11	6	13	46
No	2	11	6	13	46
Missing	0	11	1	13	8
Adjuvant Chemotherapy					
Yes	5	11	9	13	69
No	6	11	4	13	31