Original Article

Synuclein-γ in Uterine Serous Carcinoma Impacts Survival: An NRG Oncology/Gynecologic Oncology Group Study

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BACKGROUND: Synuclein-γ (SNCG) is highly expressed in advanced solid tumors, including uterine serous carcinoma (USC). The objective of the current study was to determine whether SNCG protein was associated with survival and clinical covariates using the largest existing collection of USCs from the Gynecologic Oncology Group (GOG-8023). **METHODS:** High-density tissue microarrays (TMAs) of tumor tissues from 313 patients with USC were stained by immunohistochemistry for SNCG, p53, p16, FOLR1, pERK, pAKT, ER, PR, and HER2/*neu*. Associations of SNCG and other tumor markers with overall and progression-free survival were assessed using log-rank tests and Cox proportional-hazards models, which also were adjusted for age, race, and stage. **RESULTS:** The overall survival at 5 years was 46% for women with high SNCG expression and 62% for those with low SNCG expression (log-rank $P = .021$; hazard ratio [HR], 1.31; 95% confidence interval [CI], 0.91-1.9 in adjusted Cox model). The progression-free survival rate at 5 years was worse for women who had high SNCG expression, at 40%, compared with 56% for those who had low SNCG expression (log-rank $P = .0081$; HR, 1.36; 95% CI, 0.96-1.92 in adjusted Cox model). High levels of both p53 and p16 were significantly associated with worse overall survival (p53: HR, 4.20 [95% CI, 1.54-11.45]; p16: HR, 1.95 [95% CI, 1.01-3.75]) and progression-free survival (p53: HR, 2.16 [95% CI, 1.09-4.27]; p16: HR, 1.53 [95% CI, 0.87-2.69]) compared with low levels. **CONCLUSIONS:** This largest collection of USCs to date demonstrates that SNCG was associated with poor survival in univariate analyses. SNCG does not predict survival outcome independent of p53 and p16 in models that jointly consider multiple markers. ***Cancer*** **2017;123:1144-55.** *© 2016 American Cancer Society.*

KEYWORDS: endometrial cancer, p16, p53, synuclein-γ (SNCG), uterine serous carcinoma.

INTRODUCTION

Endometrial carcinoma is the most common gynecologic malignancy in the United States, with an estimated 54,870 diagnosed in 2015.[1] Despite an overall good prognosis, the survival of women with endometrial carcinoma varies dramatically, depending upon the histologic subtype. Although uterine serous carcinoma (USC) accounts for about 10% of endometrial cancers, the prognosis is substantially worse than the more common endometrioid adenocarcinoma, with frequent

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recurrences and high mortality rates.[2,3] Active treatment modalities remain elusive, because neither its pathogenesis nor the nature of its aggressive behavior and chemoresistance is well understood.

It has been demonstrated that synuclein-γ (SNCG) is overexpressed in USC.[4-6] SNCG is a member of the synuclein family of proteins, which are small, soluble, highly conserved neuronal proteins implicated in both neurodegenerative diseases and cancer. SNCG overexpression occurs in multiple cancers, including colon, gastric, pancreatic, ovarian, and lung cancers.[7-14] SNCG was first termed breast cancer-specific gene 1 (BCSG1), because it was correlated with a poor prognosis and advanced stage in breast cancer.[7,10] The mechanisms by which SNCG promotes advanced disease and chemoresistance reportedly involve modulating the mitogen-activated kinases (MAPKs), extracellular signal-regulated protein kinases 1 and 2 (ERK1/ERK2), and c-Jun N-terminal kinase 1 (JNK1).[15] In addition, it has been demonstrated that SNCG interferes with paclitaxel-induced mitotic arrest by interacting with the mitotic checkpoint kinase BUB1B (budding uninhibited by benzimidazoles 1, beta), resulting in the inability of preventing cells with misaligned chromosomes from exiting mitosis.[16] Additional studies are necessary to define the role of SNCG in USC.

We first identified SNCG expression specifically in USC through a pathway-focused expression array, followed by correlative analysis of SNCG expression with survival in 20 patients with USC.[6] Although statistical significance was not reached because of a limited sample size, a trend toward an association of SNCG with decreased progression-free survival was evident. In a larger study evaluating 279 endometrial carcinomas with various histologies, of which 46 were USC, SNCG expression was positive in tumors from patients who had worse overall outcomes, especially those with clear cell, serous, and carcinosarcoma histologies.[5] These data strongly suggest that SNCG may be a prognostic biomarker for USC.

The objective of this study was to determine whether SNCG protein was associated with clinicopathologic variables and patient outcomes in a sufficiently large collection of USC tumors. The associations of SNCG and other tumor markers, including p53 (tumor protein 53) and p16 (cyclin-dependent kinase inhibitor 2A), with multiple clinical parameters, including survival, were determined. To our knowledge, this is the largest collection of USCs to date, representing 313 women with USC obtained from the Gynecologic Oncology Group (GOG) through its clinical trial programs.

MATERIALS AND METHODS

Patient Selection

In the GOG-8023 study, women with USC who were eligible for and enrolled in GOG-0210 (a molecular staging study[17], had consented for future research, and had histologically confirmed USC were included for tumor microarray (TMA) construction. If there was insufficient tissue submitted on GOG-0210, then tissue collected on another study, GOG-0136 (a specimen banking study), was used. The diagnosis of USC was reviewed for each case by the GOG Pathology Committee. Research specimens were reviewed by the study pathologists to confirm that primary tumor consisted of at least 90% serous carcinoma. The presence of any nonserous histologic components was noted, but the histology in all cases was considered consistent with USC overall.

Clinical Data

Overall survival was defined as the observed length of life from study entry to death. Progression was defined as increasing clinical, radiologic, or histologic evidence of disease after study entry; and progression-free survival was defined as the time from study entry to the date of disease recurrence, progression, or death (whichever occurred first). Lengths of follow-up from study entry until the date of last contact for women without death or progression were treated as censored observations for overall survival and progression-free survival analyses, respectively. Types of adjuvant therapy were recorded using the following general terms: chemotherapy, radiation therapy, chemoradiation, hormonal therapy, other treatment regimen, or none. Additional details were recorded when appropriate. Other variables examined were age (at study entry); race; International Federation of Gynecology and Obstetrics 1988 surgical stage (I-II vs III-IV); presence or absence of lymphovascular space invasion; depth of myometrial invasion (none, $< 50\%$, $\geq 50\%$, serosal involvement); involvement of pelvic and/or para-aortic lymph nodes; and presence or absence of pelvic disease, abdominal disease, peritoneal disease, and distant disease.

TMA

A high-density TMA was created by the GOG Tissue Bank, which consisted of four 10×10 grids of 0.6-mm tissue cores positioned as 4 quadrants on 1 microarray. Each 10×10 grid included 90 randomly positioned USC patient tissues as well as 10 control tissues (5 normal human tissues, 5 human cancer tissues). Of the 90 tumors, 47 were represented in duplicate for a total of 313 patient

TABLE 1. Antibody Assay Characteristics

Antibody	Source	Clone	Dilution	Antigen Retrieval
SNCG	Abcam (Cambridge, UK)	EP1539Y	1:500	TRS
ER	Dako (Carpinteria, Calif)	1D5	1:600	TRS
PR	Dako	PgR 636	1:10	TRS
p53	Dako	DO-7	1:50	TRS
HER2/*neu*	Dako	A0485 Polyclonal	1:1000	Decloaking chamber, pH6.0
p16	Ventana (Tucson, Ariz)	E6H4	1:600	CC1
p(S473)-AKT	Abcam	Polyclonal	1:100	TRS
p(Y204)-ERK	Abcam	Polyclonal	1:200	TRS
FOLR1	Leica Microsystems (Wetzlar, Germany)	BN3.2	1:50	EDTA

Abbreviations: CC1, cell condition 1; EDTA, ethylenediamine tetraacetic acid; ER, estrogen receptor; FOLR1, folate receptor 1; p(S473)-AKT, phosphorylated protein kinase B; p16, cyclin-dependent kinase inhibitor 2A gene; p53, tumor protein 53; p(Y204)-ERK, phosphorylated extracellular signal-regulated kinase; PFS, progression-free survival; PgR, progesterone receptor; PR, progesterone receptor; SNCG, synuclein-γ; TRS, target retrieval solution.

tumors represented in the TMA. There were 4 replicate TMAs.

Immunohistochemistry

Immunohistochemical (IHC) analyses were performed for the following biomarkers: SNCG, estrogen receptor (ER), progesterone receptor (PR), p53, human epidermal growth factor type 2 receptor (HER2/*neu*), folate receptor 1 (FOLR1), p16, phosphorylated protein kinase B (pAKT), and phosphorylated extracellular signal-regulated kinase (pERK). Immunostains for all except HER2/*neu* were performed at the GOG Tissue Bank housed at the Biopathology Center, which is part of the Research Institute at Nationwide Children's Hospital, under the supervision of Dr. Nilsa Ramirez. Immunostaining for HER2/*neu* was performed at the Pathology Core Facility of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University under the supervision of Dr. Jian-Jun Wei. The details for each antibody are summarized in Table 1. All antibodies were tested on negative and positive control tissues provided by both the Northwestern Human Pathology Core and the GOG Tissue Bank.

To validate the immunostains, each biomarker was also assessed in conventional blocks from 10% of the cases to confirm that the expression of each biomarker in the 0.6-mm cores was representative of the expression in full tissue sections. A semiquantitative immunoreactivity for all markers was scored by 2 pathologists. All immunostains except HER2/*neu* were scored by intensity (1+, 2+, or 3+) and by the percentage of stained tumor cells (0%, 1%-10%, 11%-20%, 21%-30%, 31%-40%, 41%-50%, 51%-60%, 61%-70%, 71%-80%, 81%-90%, 91%-100%). HER2/*neu* was scored as 0, 1+, 2+, or 3 + based on the 2007 scoring criteria established for breast cancer.[18] Marker definitions for each of the included biomarkers are delineated in Table 2. For SNCG, intensity and percentage scores were initially combined into overall scores of low, medium, and high. The *low* category was defined as either no staining (0%) or 1 + intensity with $\leq$ 20% of cell stained. The *medium* category was defined as 1 + intensity with $>$ 20% of cells stained or 2 + to 3 + intensity with from 1% to 50% of cells stained. The *high* category was defined as 2 + to 3 + intensity with $>$ 50% of cells stained. For p53, the *high* category was defined as >30% with any intensity or 0% labeling index (dead negative, indicative of null mutation).

The final immunoscores obtained for SNCG, p53, and pAKT were based on the most frequent scores from quadruplicate tissue cores. When this algorithm was inconclusive, the raw data were reviewed, and a representative summary score was determined. For the remaining markers, only 1 TMA reading was performed. Upon initial analysis for SNCG staining, the survival curves were similar for the medium and high groups. Thus, these categories were combined into a single *high* category, resulting in 2 SNCG expression groups (*low* and *high*) that were subsequently used for analysis.

Power Considerations

The study was originally designed to include 3 SNCG expression groups (low, medium, and high). Across a range of SNCG expression group distributions, a total sample size of 300 afforded 80% power, with 5% probability of a 2-sided Type I error and 10% loss-to-follow-up, to detect overall survival hazard ratios (HRs) from 0.46 to 0.56 for low versus medium SNCG expression and from 1.63 to 1.81 for high versus medium SNCG expression, depending on the size of the low, medium, and high groups.[19] A target sample size of 360 for TMA construction was set to

TABLE 2. Marker Expression Pattern Definitions

Marker	Expression Pattern Definitions: % (Intensity)			Expression Patterns Included
	High Expression	Medium Expression	Low Expression	
SNCG	>20% (2+/3+)	>20% (1+) *or* 1%-50% (2+/3+)	≤20% (0/1+)	Cytoplasmic and nuclear
ER	>10% (Any intensity)	NA	0% *or* ≤10% (Any intensity)	Nuclear
PR	>10% (Any intensity)	NA	0%, *or* ≤10% (Any intensity)	Nuclear
p53	>30% (Any intensity) *or* 0% labeling index	NA	1%-30% (Any intensity)	Nuclear
p16	>50% (Any intensity)	NA	≤50% (Any intensity)	Nuclear and cytoplasmic
pAKT	>50%, (1+) *or* >20% (2+/3+)	NA	≤50% (1+) *or* ≤20% (2+/3+)	Membranous and cytoplasmic
pERK	>50% (1+) *or* >20% (2+/3+)	NA	≤50% (1+), *or* ≤20% (2+/3+)	Nuclear and cytoplasmic
FOLR1	>10% (Any intensity)	NA	≤10% (Any intensity)	Membranous
HER2/*neu*[a]	Positive (score 3+)	Equivocal (score 2+)	Negative (score 0 or 1+)	Membranous

Abbreviations: ER, estrogen receptor; FOLR1, folate receptor 1; Her2/*neu*, human epidermal growth factor type 2 receptor; NA, not applicable; p16, cyclin-dependent kinase inhibitor 2A; p53, tumor protein 53; pAKT, phosphorylated protein kinase B; pERK, phosphorylated extracellular signal-regulated kinase; PR, progesterone receptor; SNCG, synuclein-γ.

[a] Scoring for Her2/*neu* was based on the 2007 scoring criteria recommended for breast cancer (Pan ZZ, Bruening W, Giasson BI, Lee VM, Godwin AK. Gamma-synuclein promotes cancer cell survival and inhibits stress- and chemotherapy drug-induced apoptosis by modulating MAPK pathways. *J Biol Chem*. 2002;277:35050-35060[15]).

allow for potential core loss. In our analyses, because of the similarity of effect estimates, the medium and high expression groups were combined for analysis. In post hoc power calculations, our observed sample sizes in the SNCG expression groups yielded 80% power with 5% probability of a 2-sided Type I error to detect an HR of roughly 1.62 for the high versus low SNCG expression groups.

Statistical Analysis

Clinical and biomarker variables were summarized using means and standard deviations for age and tables of frequencies and counts for all other categorical variables. SNCG expression was the primary predictor of interest. Analyses were initially conducted using 3 SNCG expression categories, as planned. Few differences in survival distributions and hazard functions were observed in all analyses for the original medium and high expression groups; hence, these 2 categories were combined, and 2 SNCG expression groups (high vs low) were used for final analyses (Table 3). Secondary predictors of interest were expression of FOLR1, pERK, pAKT, p53, p16, ER, and PR (all high vs low) as well as HER2/*neu* expression (positive, negative, or equivocal). The primary outcome was overall survival (time in months), and the secondary outcome was progression-free survival (time in months).

Age, race, surgical stage, presence of lymph-vascular space invasion, depth of myometrial invasion, pelvic and/or para-aortic lymph node involvement, pelvic disease, abdominal disease, peritoneal disease, distant disease, and adjuvant treatment were all summarized to describe the patient population and were examined for associations with SNCG expression using a t test for age and a chi-square or Fisher exact test for categorical variables. Clinical characteristics that demonstrated an association with SNCG expression groups at $P < .05$ were included in Cox proportional hazards models to assess potential confounding in SNCG associations with time-to-event outcomes.

Kaplan-Meier curves for overall and progression-free survival were generated for both SNCG groups. Log-rank tests were used to assess differences between the curves. Cox proportional hazards models were used to estimate HRs, and adjustments for age, race, and disease stage were examined in Cox models. The same process was used for all tumor markers of secondary interest. HRs were estimated in multiple marker models for SNCG and p16 and for SNCG and p53.

RESULTS

SNCG in USC

USC tumors from patients enrolled on GOG-0210 were collected, and TMAs were constructed by the GOG Tissue Bank (available at: http://www.nationwidechildrens.org/biopathology-center-collaborations). Clinical data and adequate tissue specimens were available for analysis from 313 patients. Immunostaining for SNCG revealed a variable extent (focal/patchy to extensive/diffuse) and intensity of staining, which was localized predominantly to the cytoplasm of tumor cells, with occasional nuclear staining (Fig. 1). The staining was categorized as high or low based on the intensity of staining and the percentage of cells stained (low, 0-1 intensity with ≤ 20% of tumor cells stained; high, 2-3 intensity or > 20% of tumor cells

stained) (Table 2). High expression of SNCG was observed in 61.8% of specimens (Table 3). There was a statistically significance difference in the mean age at diagnosis, which was 67.2 years and 69.8 years for the low and high SNCG expression groups, respectively ($P = .01$) (Table 4). Surgical stage; histologic heterogeneity; the presence of lymphovascular space invasion; the depth of myometrial invasion; pelvic and para-aortic lymph node metastasis; the presence of pelvic, abdominal, peritoneal, or distant disease; and the type of adjuvant treatments received were similar across the SNCG groups. Although no differences in SNCG expression were observed according to race or stage, these covariates were included along with age in adjusted models because of their known associations with overall survival.

TABLE 3. Marker Expression Frequencies

Marker	Expression: No. (%)		
	Low	High	Total
SNCG	115 (38.2)	186 (61.8)	301
p53	42 (13.6)	267 (86.4)	309
p16	35 (12.2)	252 (87.8)	287
FOLR1	104 (37)	177 (63)	281
pERK	237 (82)	52 (18)	289
pAKT	264 (85.7)	44 (14.3)	308
PR	184 (63.5)	106 (36.6)	290
ER	171 (59.2)	118 (40.8)	289
HER2/*neu*[a]			313
Negative	299 (95.5)	—	
Equivocal	7 (2.2)	—	
Positive	7 (2.2)	—	

Abbreviations: ER, estrogen receptor; FOLR1, folate receptor 1; Her2/*neu*, human epidermal growth factor type 2 receptor; NA, not applicable; p16, cyclin-dependent kinase inhibitor 2A; p53, tumor protein 53; pAKT, phosphorylated protein kinase B; pERK, phosphorylated extracellular signal-regulated kinase; PR, progesterone receptor; SNCG, synuclein-γ.
[a] Because the sample sizes for HER2/*neu* were too small, survival analyses are not reported.

In unadjusted analyses, overall survival was statistically significantly worse for women who had tumors that demonstrated high SNCG expression (log-rank test; $P = .021$) (Fig. 2, Table 5). At 5 years, the overall survival estimates were 62% for the low SNCG expression group and 46% for the high SNCG expression group. The association between SNCG and overall survival was attenuated in adjusted Cox models, with an HR of 1.31 (95% confidence interval [CI], 0.91-1.9; $P = .15$) after adjusting for age, race, and stage. Progression-free survival was

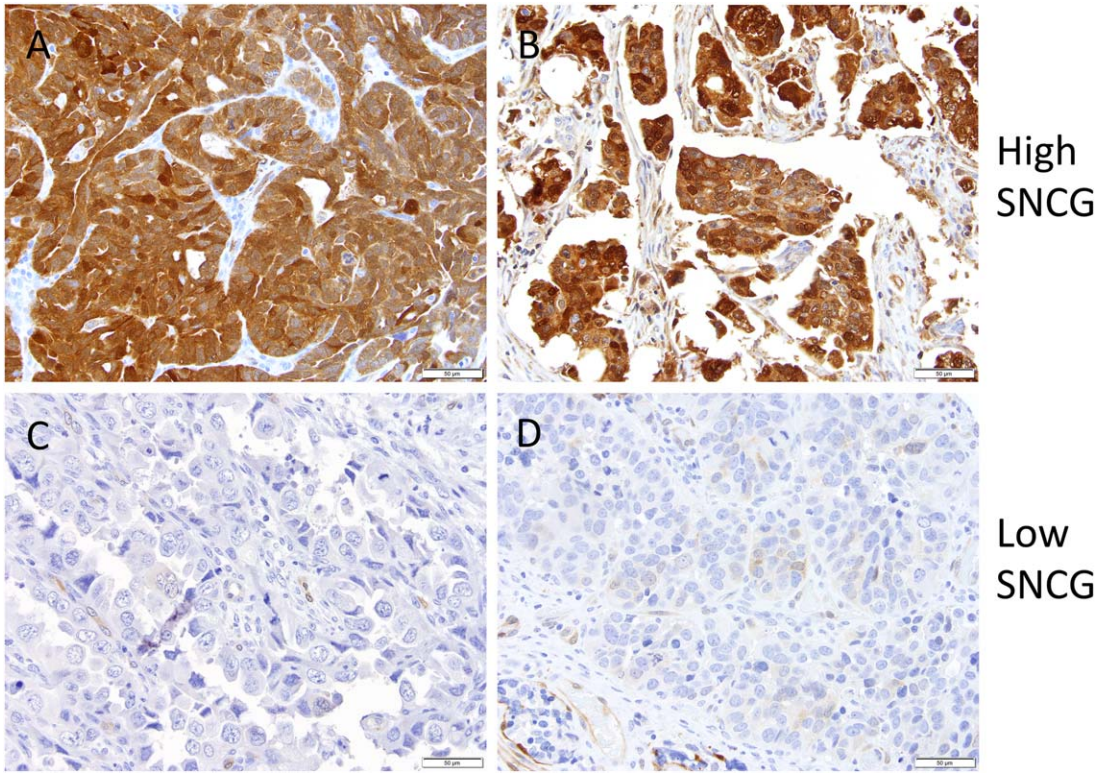

Figure 1. (A-D) Immunohistochemical staining for synuclein-γ (SNCG) is observed in tumor cores from the uterine serous carcinoma tissue microarray. Two representative sections of (A,B) high expression and (C,D) low expression are shown. Brown color represents positive staining for SNCG.

TABLE 4. Patient Characteristics

Characteristic	No. of Patients (%)			
		SNCG Expression		
	All Patients, n = 313	Low, n = 115	High, n = 186	P^a
Age: Mean ± SD, y	68.7 ± 8.6	67.2 ± 9.0	69.8 ± 8.1	.01
Race				
White	217 (74.3)	88 (79.3)	129 (71.3)	.33
Black	70 (24)	22 (19.8)	48 (26.5)	
Other	5 (1.7)	1 (0.9)	4 (2.2)	
FIGO 1988 surgical stage				
1-2	154 (49.2)	61 (53)	85 (45.7)	.22
3-4	159 (50.8)	54 (47)	101 (54.3)	
Diagnostic pathology review classification				
Pure serous carcinoma	239 (76.4)	83 (72.3)	145 (78)	.18
Serous with endometrioid features, indeterminate	42 (13.4)	15 (13)	26 (14)	
Other	32 (10.2)	17 (14.8)	15 (8.1)	
Malignant cells in vascular lymphatic space				
Absent	169 (55)	65 (58)	97 (53)	.40
Present	138 (45)	47 (42)	86 (47)	
Depth of myometrial invasion				
None	64 (20.9)	16 (14.2)	42 (23.1)	.25
<50%	119 (38.8)	50 (44.3)	66 (36.3)	
>50%	105 (34.2)	41 (36.3)	63 (34.6)	
Serosa	19 (6.2)	6 (5.3)	11 (6)	
Pelvic and/or para-aortic lymph node metastasis				
None	177 (63.2)	76 (69.7)	101 (59.1)	.15
Pelvic only	37 (13.2)	10 (9.2)	27 (15.8)	
Para-aortic with or without positive pelvic lymph nodes	66 (23.6)	23 (21.1)	43 (25.2)	
Pelvic disease				
No	210 (71.4)	87 (77.7)	123 (67.6)	.06
Yes	84 (28.6)	25 (22.3)	59 (32.4)	
Abdominal disease				
No	222 (81.6)	85 (86.7)	137 (78.7)	.10
Yes	50 (18.4)	13 (13.3)	37 (21.3)	
Peritoneal disease				
No	210 (70.2)	86 (74.8)	124 (67.4)	.17
Yes	89 (29.8)	29 (25.2)	60 (32.6)	
Distant disease				
No	151 (98)	54 (98.2)	91 (97.9)	1.00
Yes	3 (2)	1 (1.8)	2 (2.2)	
Adjuvant treatment				
Chemotherapy	122 (51.1)	46 (50)	70 (51.1)	.40
Radiation	27 (11.3)	8 (8.7)	19 (13.9)	
Chemotherapy and radiation	90 (37.7)	38 (41.3)	48 (35)	

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; SD, standard deviation; SNCG, synuclein-γ.
[a] *P* values are for comparisons of characteristics across low and high SNCG expression groups. The Student *t* test was used for comparison of age. All other categorical comparisons were evaluated using chi-square tests, except for depth of myometrial invasion and distant disease, which used the Fisher exact test.

also statistically significantly lower for women who had tumors with high SNCG expression in unadjusted analyses (log-rank test; $P = .0081$) (Fig. 2, Table 5). At 3 years, 63% of women who had tumors with low SNCG expression were progression free versus 47% of those who had tumors with high SNCG expression. This also was observed at 5 years, with progression-free survival rates of 56% and 40% for the low and high SNCG expression groups, respectively. A Cox model HR adjusted for age, race, and stage favored low SNCG expression (HR for low vs high expression, 1.36; 95% CI, 0.96-1.92; $P = .086$). The Kaplan-Meier plots in Figure 2 illustrate lower survival for the high SNCG group compared with the low SNCG expression group. The trends were similar when survival was examined separately for white and black (Supporting Fig. 1; see online supporting information).

Association of Other Tumor Markers in USC

Next, we sought to determine the expression patterns of other known molecular markers in endometrial cancer and their associations with progression and survival outcomes. The TMAs were stained for p53, p16, FOLR1,

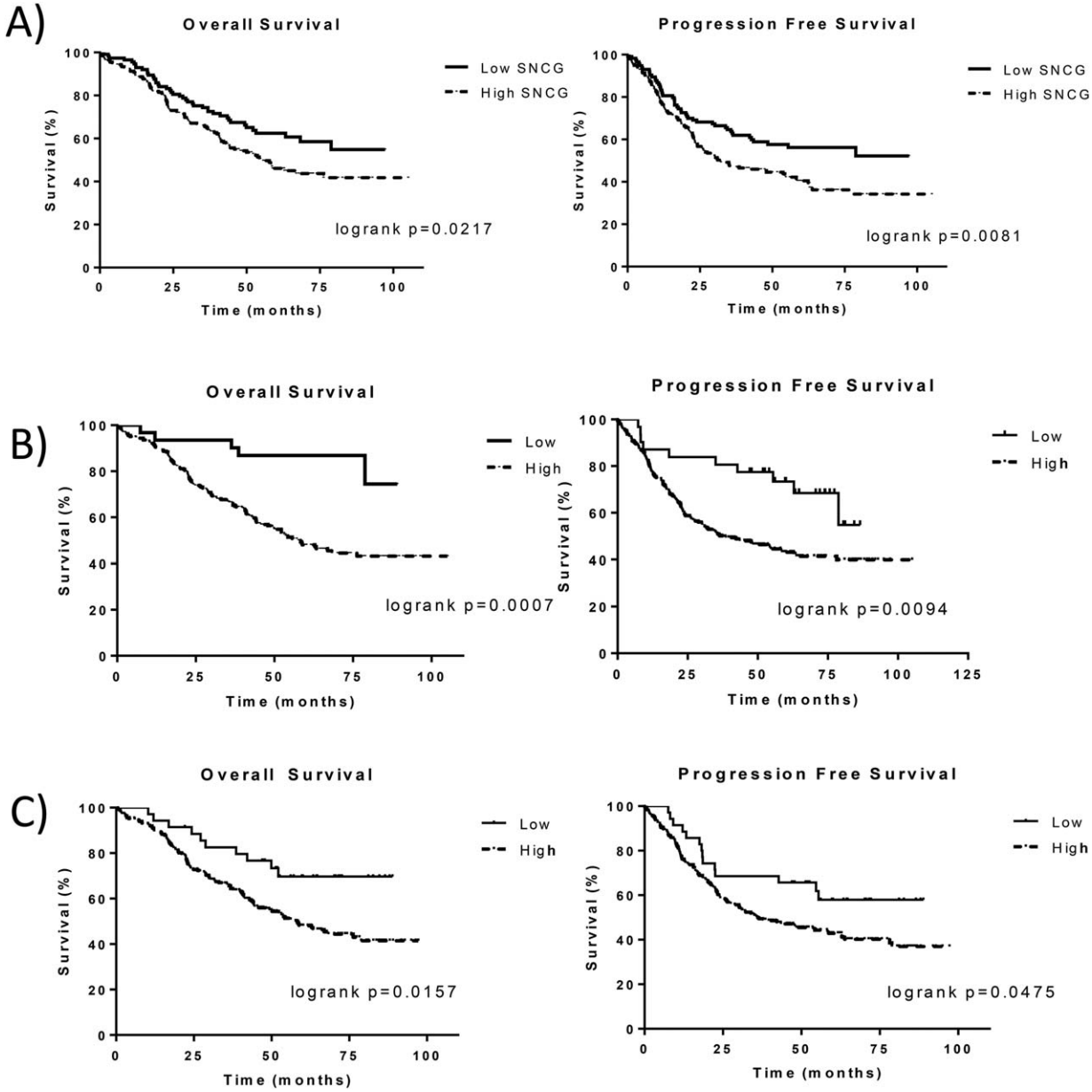

Figure 2. (A-C) Kaplan-Meier curves illustrate the survival of patients with uterine serous carcinoma stratified according to (A) synuclein-γ (SNCG) expression, (B) p53 expression, and (C) p16 expression (high vs low). Statistically and clinically significant differences were observed between the groups for both overall and progression-free survival.

pERK, pAKT, PR, ER, and HER2/*neu*, and their expression levels were scored as either high or low (Table 3, Supporting Figs. 2 and 3; see online supporting information). Greater than 50% of patients had high immunoreactivity for SNCG, p53, p16, and FOLR1, as summarized in Table 3. HER2/*neu* was positive in only 2.2% of USC samples and was negative in >95%. Less than 20% of samples exhibited high immunoreactivity for pERK and pAKT, whereas the majority (>80%) had low levels. Immunoreactivity for both ER and PR was low in more than 50% of samples.

Among the markers tested, only p53 and p16 were significantly associated with unfavorable clinical outcomes. Women who had tumors that demonstrated high p53 expression had worse overall survival (HR, 4.2; 95% CI, 1.54-11.45; $P = .005$) and disease progression (HR, 2.16; 95% CI, 1.09-4.27; $P = .027$) (Table 6). Trends were also evident for those who had tumors with high p16

TABLE 5. Synuclein-γ Expression and Survival Estimates

SNCG Expression	No. (No. of Events)	Survival[a]			Log-Rank *P*	HR [95% CI][b]	*P*
		1 Year	3 Years	5 Years			
OS						1.31 [0.91-1.9]	.15
High	186 (93)	0.89	0.66	0.46	.021		
Low	115 (43)	0.94	0.73	0.62			
PFS						1.36 [0.96-1.92]	.086
High	186 (109)	0.77	0.47	0.40	.0081		
Low	115 (49)	0.81	0.63	0.56			

Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; SNCG, synuclein-γ.
[a] OS and PFS estimates were evaluated based on high and low SNCG expression levels using the log-rank test.
[b] HRs were calculated by comparing high versus low SNCG expression and are adjusted for age, race, and stage with 95% CIs and *P* values.

expression associated with worse overall survival (HR, 1.95; 95% CI, 1.01-3.75; $P = .046$) and progression-free survival (HR, 1.53; 95% CI, 0.87-2.69; $P = .14$) (Table 6). Expression levels of p16 and p53 were associated, with approximately 92% of tumors that had high p53 expression also demonstrating high p16 expression ($P < .0001$). Cox models using multiple markers were also examined to determine whether the association of SNCG with overall and progression-free survival was independent of p53 and p16 associations. In Cox models that included SNCG and p53 as well as age, race, and stage, associations of SNCG with the outcomes were attenuated and were no longer statistically significant, with an HR of 1.19 (95% CI, 0.81-1.73; $P = .37$) for overall survival and 1.27 (95% CI, 0.89-1.81; $P = .19$) for progression-free survival. When SNCG and p16 were included in a model together, associations with overall and progression-free survival were attenuated and were not statistically significant for either marker. Nevertheless, > 90% of tumors with high SNCG expression also had high p53 and/or p16 expression (Table 7). The expression of FOLR1, pERK, pAKT, PR, and ER and the HRs for these markers were not statistically significant (Table 8).

DISCUSSION

The incidence of USC is rare, accounting for only 10% of newly diagnosed endometrial cancers. However, USC is one of the most aggressive tumors of the endometrium, with high recurrence and associated mortality rates.[2,3] Active treatment modalities remain elusive, because neither its pathogenesis nor its chemoresistance is well understood. From one-third to one-half of USC tumors are admixed with other histologic subtypes,[20] although recent literature based on data from The Cancer Genome Atlas indicates that the morphologic reproducibility of carcinomas with mixed or ambiguous histology is poor, and polymerase ε (*POLE*)-ultramutated endometrioid carcinomas in particular may be morphologically misdiagnosed as USC.[21,22] Notwithstanding these newer data, however, morphology-based studies have indicated that, even when the USC component contributes as little as 10% to the tumor, its behavior can resemble pure serous carcinoma.[23] A significant limitation to studying USC is the small number of patients at any one institution. The GOG, with the cooperation of multiple centers, has collected thousands of endometrial cancer samples through various clinical trial protocols. Specifically, the USC tumor specimens used in the current study were collected and banked as part of the GOG-0210 and GOG-0136 clinical trials. Consequently, 313 patients with USC who had adequate tumors represented on the TMA and detailed clinical information were available for this study, representing the largest collection of USC tumors with corresponding clinical information available for investigation to date. The statistical study design planned the sample size to have 80% statistical power, with 5% probability of a 2-sided Type I error and 10% loss to follow-up, to detect HRs of 0.46 to 0.56 for low versus medium SNCG expression and 1.63 to 1.81 for high versus medium SNCG expression in the original 3-group design. The results revealed a statistically significant association between SNCG expression and both overall and progression-free survival in univariate analyses. In addition, given the size of this cohort, standardized criteria for a relatively reliable cutoff score for SNCG to allow for interpretation of immunoreactivity for SNCG could be established. Consistent with a recent study,[4] scoring for low and high SNCG expression is a reproducible approach to interpreting IHC scores for SNCG in USC. Further validation of technical methodology and interpretation criteria will be needed before the widespread adoption of SNCG IHC staining as a diagnostic or prognostic marker.

According to our study, the survival of women who had tumors with high SNCG expression was worse, despite the absence of a statistically significant association

TABLE 6. Correlation of p53 and p16 Expression With Survival

Marker Expression	No. of Events (%)	Survival[a]			Log-Rank *P*	HR [95% CI]	*P*
		1 Year	3 Years	5 Years			
OS						4.20 [1.54-11.45]	.005
p53							
High	267 (130)	0.90	0.66	0.48	.0008		
Low	42 (9)	0.95	0.86	0.80			
p16						1.95 [1.01-3.75]	.046
High	252 (124)	0.90	0.66	0.48	.016		
Low	35 (10)	0.94	0.83	0.70			
PFS							
p53						2.16 [1.09-4.27]	.027
High	267 (147)	0.78	0.50	0.42	.0092		
Low	42 (15)	0.81	0.76	0.71			
p16						1.53 [0.87-2.69]	.14
High	252 (141)	0.76	0.50	0.43	.048		
Low	35 (14)	0.89	0.69	0.58			

Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival; p16, cyclin-dependent kinase inhibitor 2A; p53, tumor protein 53.
[a] OS and PFS estimates were based on high and low p53 and p16 expression using the log-rank test.
[b] HRs were calculated by comparing high versus low expression for each marker and were adjusted for age, race, and stage with 95% CIs and *P* values.

TABLE 7. Associations Between Significant Markers[a]

	p53: No. (%)			p16: No. (%)			p16: No. (%)	
SNCG	Low	High	SNCG	Low	High	p53	Low	High
Low	23 (20)	92 (80)	Low	24 (22)	84 (78)	Low	19 (54)	16 (46)
High	16 (9)	170 (91)	High	11 (6)	168 (94)	High	19 (8)	233 (92)
P		.00042			< .0001			< .0001

Abbreviations: p16, cyclin-dependent kinase inhibitor 2A; p53, tumor protein 53;SNCG, synuclein-γ.
[a] Data represent counts, with row percentages shown in parentheses. *P* values indicate whether the association between high and low expression of *pairs* of markers was statistically significant.

TABLE 8. Hazard Ratios for the Additional Markers

Variable	HR (95% CI)[a]				
	FOLR1	pERK	pAKT	PR	ER
OS	1.04 (0.72-1.51)	1.10 (0.68-1.76)	1.00 (0.63-1.58)	0.82 (0.57-1.19)	0.92 (0.65-1.32)
P	.84	.70	.99	.30	.66
PFS	1.16 (0.81-1.64)	1.22 (0.80-1.85)	1.09 (0.73-1.65)	0.78 (0.55-1.09)	0.90 (0.65-1.25)
P	.42	.37	.67	.15	.52

Abbreviations: CI, confidence interval; ER, estrogen receptor; FOLR1, folate receptor 1; HR, hazard ratio; OS, overall survival; pAKT, phosphorylated protein kinase B; pERK, phosphorylated extracellular signal-regulated kinase; PFS, progression-free survival; PR, progesterone receptor.
[a] HRs were calculated by comparing high versus low expression for each marker and are adjusted for age, race, and stage with 95% CIs and *P* values.

between SNCG expression and certain clinical parameters at the time of USC diagnosis, including stage, depth of myometrial invasion, lymphovascular space invasion, and lymph node metastasis. Unadjusted analyses demonstrated a statistically significant association between SNCG expression and both overall and progression-free survival, although the associations were attenuated after adjustment for age, race, and stage. Additional investigations with larger samples may clarify these associations, because the observed HRs for both time-to-event outcomes in these data were slightly lower than the HRs we were adequately powered to detect. USC is an aggressive malignancy, with early intra-abdominal and retroperitoneal spread even in the absence of traditional risk factors, such as deep myometrial invasion, tumor size, and lymphovascular space invasion.[3] Thus SNCG may be associated with

mechanisms related to this unique spread pattern. However, there is still much to be learned regarding the genes and pathways that permit metastasis preferentially into the abdominal and peritoneal cavities.

Molecular studies have suggested the involvement of SNCG in chemoresistance. It was demonstrated that SNCG binds to a spindle checkpoint kinase, BUB1B, thereby inducing a structural change in BUB1B. This inhibited its kinase activity and attenuated its interaction with other key checkpoint proteins, such as cell-division cycle protein 20 (Cdc20), compromising the spindle assembly checkpoint.[16,24,25] The lack of checkpoint function would allow cells to override G2/M arrest with aneuploidy proliferation to perpetuate genomic instability. By targeting SNCG with a specific peptide, sensitivity to paclitaxel was enhanced.[26] The association of SNCG with clinical chemoresistance was not assessed in the current study because of insufficient information, and it remains an unanswered question. Most women in this study received some form of adjuvant therapy, and the distribution of chemotherapy, pelvic radiotherapy, or both was similar in both the low and high SNCG expression groups. The use of SNCG as a marker for chemoresistance is a plausible option that should be explored.

We did examine the association of SNCG with other tumor markers in this study. Among the markers tested, only p53 and p16 were associated with both overall and progression-free survival. To our knowledge, this is the largest cohort to demonstrate the association of p53 or p16 with survival in patients with USC, providing evidence of the prognostic potential of these 2 markers. Only one other study demonstrated a significant association of p53 with worse survival in 34 patients with USC,[27] whereas association studies of p16 and survival in USC have not been reported, underscoring the relevance of our study of 313 women with USC. High versus low expression of the other markers (pAKT, pERK, ER, PR, and FOLR1) was not significantly associated with survival. The expression of these markers has been studied extensively in endometrioid carcinoma, but their role in USC is less understood. ER has been associated with SNCG, which increases the transcriptional activity of ER to mediate estrogen-driven proliferation in the mammary gland.[28-30] SNCG can stimulate membrane-initiated estrogen signaling to stimulate growth and promote tamoxifen resistance in breast cancer cells.[30] The data regarding whether ER contributes to the aggressive nature of USC are sparse, although USC is distinct from endometrioid adenocarcinoma with regard to hormone dependence. An analysis of 71 women with USC in Japan demonstrated an overall and progression-free survival advantage with positive hormone receptor status.[31] However, in our study, neither ER nor PR was associated with survival outcomes. In our study, we grouped IHC staining for ER and PR according to high versus low expression, whereas the Japanese study compared positive hormone (either ER or PR) expression versus negative hormone expression (for either ER or PR). Nevertheless, in our study, 40% and 36% of USC tumors expressed high levels of ER and PR, respectively. It remains to be determined whether ER and PR actively influence the behavior of USC. Signaling pathways, including AKT and MAPK, have been implicated in driving metastasis and chemoresistance in tumors[32-36] and thus were markers of interest in the current study. Moreover, it has been demonstrated that SNCG maintains pAKT and mechanistic target of rapamycin (mTOR) activities that protect cells from the cytotoxicity related to disabling heat-shock protein 90 (Hsp90).[37] Similarly, SNCG protects HER2/*neu* function, rendering it resistant to Hsp90-mediated toxicity.[38] In the current study, although none of these markers were independently associated with survival, it is possible that they may play a role in resistance to treatments.

HER2/*neu* is amplified in a wide range of tumors, from 10% to 65%, depending on the study.[39-42] In our study, staining for HER2/*neu* was low. It is noteworthy that we used breast cancer criteria for scoring HER2/*neu* expression in USC, because specific criteria for scoring HER2/*neu* in USC have not yet been established. One study reported that screening for HER2/*neu* with IHC overestimated the number of tumors with HER2/*neu* gene amplification, because there was significant discordance between IHC and in situ hybridization results.[43] In addition, the clinical relevance of HER2/*neu* in USC is not entirely clear: Some studies have reported an association between HER2/*neu* and poor overall survival in patients with Type II endometrial cancer (and specifically USC),[39,44] whereas others have demonstrated no association with survival.[41] We could not conduct a survival analysis for HER2/*neu* expression in our study because of the low numbers of tumors that exhibited staining. Additional testing with in situ hybridization staining along with IHC would be a more accurate measure of the positive cases.

In summary, this study demonstrated a statistically significant association of SNCG with poor survival outcomes among patients with USC in unadjusted analyses, although some attenuation of the association was observed after adjustment for age, race, and stage. Levels of p53 and p16 also were associated significantly with worse survival. In analyses of multiple markers, SNCG did not demonstrate statistically significant associations after adjustment

for p53 or p16; hence, these data do not support SNCG as an independent predictor of survival outcomes. However, the association of SNCG with markers of advanced disease merits further investigation of its role in USC biology or as a predictive biomarker. Unlike p53 or p16, SNCG has been detected in sera from patients who harbor tumors.[9,14,45,46] A serum biomarker, along with other tumor markers, could aid in earlier diagnosis or detecting recurrence. In addition, the role of SNCG in predicting chemoresistance remains to be studied. Finally, this study reports the largest collection of patients with USC who had clinical information indicating that SNCG, p53, and p16 are associated with worse survival outcomes. This resource can be used to study other promising tumor marker candidates for this rare uterine cancer.

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