GPR30 Predicts Poor Survival for Ovarian Cancer

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Abstract

Objectives—GPR30 is a 7-transmembrane G protein-coupled estrogen receptor that functions alongside traditional estrogen receptors to regulate cellular responses to estrogen. Recent studies suggest that GPR30 expression is linked to lower survival rates in endometrial and breast cancer. This study was conducted to evaluate GPR30 expression in ovarian tumors.

Methods—GPR30 expression was analyzed using immunohistochemistry and archival specimens from 45 patients with ovarian tumors of low malignant potential (LMP) and 89 patients with epithelial ovarian cancer (EOC). Expression, defined as above or below the median (intensity times the percentage of positive epithelial cells) was correlated with predictors of adverse outcome and survival.

Results—GPR30 expression above the median was observed more frequently in EOC than in LMP tumors (48.3% vs. 20%, *p= 0.002*), and in EOC was associated with lower 5-yr survival rates (44.2% vs. 82.6%, Log rank *p < 0.001*). Tumor grade and FIGO stage, the other significant predictors of survival, were used to stratify cases into "high-risk" and "low risk" groups. The 5-yr survival rate for "low risk" EOC (all grade 1 and stage I/II, grade 2) was 100%. In "high risk" EOC (all grade 3 and stage III/IV, grade 2), the difference in 5-year survival by GPR 30 expression was significant (33.3% vs. 72.4%, *p = 0.001*).

Conclusions—The novel estrogen-responsive receptor GPR30 is preferentially expressed in "high risk" EOC and is associated with lower survival rates. Further investigation of GPR30 as a potential target for therapeutic intervention in high risk EOC is warranted.

Keywords

GPR30; epithelial ovarian carcinoma; low malignant potential; overall survival

INTRODUCTION

In the United States, epithelial ovarian cancer (EOC) is the 8th most common malignancy in women, with an estimated 22,430 new cases and 15,530 deaths expected in 2008 [1]. Although trends analyses using Surveillance, Epidemiology, and End Results (SEER) data indicate a steady improvement in survival rates [37% (1975–1997) vs. 45% (1996–2003)], the majority of patients present with advanced stage and ultimately die from the disease [1,2].

Recent data support a 2-tier grading system for serous ovarian cancers. Grade 1 tumors, which comprise the low-risk group, appear to arise within ovarian tumors of low malignant potential (LMP) in up to 60% of cases, occur in younger women, and have a more favorable prognosis [3–5]. The largest series to date comparing EOC by tumor grade was performed using SEER data. In this study, those with grade 1 tumors had 24- and 60-month survival rates of 88% and 70%, respectively, whereas those with grade 2 tumors had corresponding survival rates of 75% and 45%, and those with grade 3 tumors, 70% and 37%, respectively [4].

LMP tumors account for 10–15% of all EOC, are characterized by atypical nuclear features, an absence of destructive stromal invasion, and have an excellent survival rate. Whether or

not LMP tumors are a precursor to EOC is controversial. However, recent molecular profiing and genetic studies support a dualistic model for the development of serous tumors, where serous LMP tumors represent precursors of low grade serous carcinomas but high grade tumors arise "de novo" from *in situ* alterations in epithelial inclusion cysts [3–5]. Gene expression profiling studies indicate that the majority of low grade EOC tumors cluster with LMP tumors [6–8]. LMP and low-grade EOC both exhibit a high frequency of mutations in KRAS and BRAF, which are uncommon in high grade tumors [9], whereas high grade lesions exhibit greater chromosomal instability, activation of genes associated with proliferation, and down-regulation of genes that inhibit cell migration and invasion [6,10]. Thus, clinical and genetic studies support the hypothesis that LMP tumors, or at least an intermediate risk subset, termed micropapillary serous carcinoma, give rise to low-grade invasive ovarian cancer [11].

Estrogen plays a critical role in breast, endometrial, and ovarian physiology. Although the role of estrogen in ovarian carcinogenesis is less clear, tissue expression profiles of ERα show prognostic value in EOC. In a recent study of 773 ovarian tumors, ERα positivity was observed in 16% of LMP and 36% of EOC tumors; occurred more often in serous than mucinous tumors, and conferred more favorable prognosis [12]. Low grade tumors express significantly higher levels of ER, PR, and E-cadherin than do high grade tumors, suggesting their involvement in low grade EOC and LMP tumors [13]. However, although up to 60% of ovarian cancers are ERα positive, the concordance between receptor status and response to antiestrogen therapy is substantially lower for ovarian compared to breast cancer [14–16]. These findings suggest that estrogen mediates its effects on ovarian tumors at least in part through mechanisms independent of classical estrogen receptors.

We and others have described a novel intracellular 7-transmembrane G protein-coupled estrogen receptor (GPR30) that functions alongside the traditional estrogen receptors (ERα and ERβ) to regulate physiological responsiveness to estrogen [17,18]. GPR30 is stimulated by estrogen, tamoxifen, and fulvestrant to activate numerous cell signaling pathways including calcium mobilization, adenylyl cyclase, MAP kinase and phosphatidyl inositol 3-kinase, in large part via the transactivation of epidermal growth factor receptors (EGFRs) [18–21]. GPR30 is widely expressed in numerous tissues throughout the body and is often highly expressed in cancer cell lines, particularly those from aggressive tumors [18,22,23], and has been shown to be an important prognostic factor in breast and endometrial cancers [22,23]. In endometrial cancer, we reported that high levels of GPR30 expression were observed more frequently in tumors associated with lower overall survival rates when stratified by stage of disease. Moreover, GPR30 expression was inversely correlated with ER and PR expression, yet correlated positively with EGFR expression [22]. From these observations, we hypothesized that GPR30 is an alternative estrogen receptor that is overexpressed in high-risk endometrial carcinomas where expression of ER and PR is downregulated.

In ovarian cancer cell lines, it has been shown that GPR30 is able to mediate gene expression changes and proliferation in response to 17β-estradiol and the selective GPR30 agonist G-1 [24]. These observations led to the hypothesis that GPR30 activation may represent an alternative pathway for estrogen-mediated activity in high grade and advanced stage epithelial ovarian tumors that are more often ER negative, whereas LMP tumors and grade 1 tumors, which are associated with good prognosis, exhibit low levels of GPR30 expression. The purpose of the current study was to evaluate GPR30 expression using immunohistochemistry in a variety of epithelial ovarian tumors in order to elucidate potential relationships between GPR30 expression, clinical/pathological findings, and overall survival.

Material and Methods

Specimens

This study was reviewed and approved by the Institutional Review Board of the University of New Mexico Health Science Center. Paraffin blocks from 134 patients with either EOC (89) or LMP tumors (45) who underwent consultation and/or treatment at the University of New Mexico Health Sciences Center between March, 1996 and June, 2005 were retrieved from the University of New Mexico Human Tissue Repository. Available clinical data, survival time in months, and cause of death were abstracted from the University of New Mexico Hospital and Cancer Center Tumor Registry database, and these data were ultimately linked in a blinded fashion to the IHC results. The medical records were also reviewed to confirm stage of disease, survival time, and cause of death. Trained abstractors had previously assigned International Classification of Diseases for Oncology (ICD-O) codes to each case [25]. The SEER definitions of race and ethnicity were used to classify patients. A secondary pathology review was also performed in all cases by two independent observers (NEJ, HOS), and where there was discordance in the classification (11 cases), joint review was performed to reach a consensus regarding the final pathological diagnosis. In 13 cases with mixed epithelial histology but where a single histological type accounted for 75% of the morphologic appearance, the predominating histology was used to assign cell type. LMP tumors were assigned a grade of 0.

Immunohistochemistry

Five-micron sections from paraffin-embedded tumor tissues were prepared for immunohistochemistry (IHC), which was performed as previously described using a rabbit polyclonal affinity purified antibody directed against the C-terminus of GPR30 [22]. In addition, 8 cases were evaluated for GPR30 expression using a rabbit polyclonal antibody directed against the N-terminus. In brief, sections were deparaffinized in CitriSolv clearing agent (Fisher, Pittsburgh, PA) followed by dehydration in ethanol. Antigen retrieval was accomplished by microwaving slides in 0.01 M citrate buffer (pH 6.0) for 25 minutes, followed by incubation of cooled slides in fresh 2% H_2O_2 for 10 minutes. Permeabilization and blocking were performed by incubating the slides for 30 minutes in 200 μL of 0.1% Triton X-100 in PBS with 3% bovine serum albumin in a humid chamber. Slides were incubated with the GPR30 C-terminal antibody diluted 1:400 (for a final protein concentration of 2 μg/mL) or isotype control (diluted to the same final antibody protein concentration) in 3% normal goat serum for 1 hour. Following multiple washes, bound antibody was detected using the immunoperoxidase system by incubating with goat anti-rabbit IgG conjugated to horseradish peroxidase (diluted 1:250 in 3% normal goat serum) for 45 minutes. Bound peroxidase was detected by staining with the enzyme substrate 3′, 3-diaminobenzidine tetrahydrochloride (DAB; Sigma, St Louis, MO). All series of immunostaining assays included endometrial tissue known to express GPR30 as a positive control, and tissues that express GPR30 incubated with matched isotypic antibody as the negative control.

Grading of epithelial cell cytoplasmic staining was performed using an H-scoring system, obtained by multiplying the epithelial cell intensity (graded as 0 negative, 1+ weak, 2+ moderate, or 3+ strong) by the percentage of positive epithelial cells (0 –100%). For statistical analysis as well as to reduce intra-observer variability, the samples were grouped into low-expressing (H values below or equal to the median, GPR30-Low) and high-expressing (H values greater than the median, GPR30-High). In 37 cases, slides were reviewed and analyses by two examiners (HOS, NEJ). A strong positive correlation (Spearman r = 0.8, *p < 0.001*), and good agreement between their scores ($\kappa = 0.69 \pm 0.13$) was observed when the H-score of 105 (the median of EOC) was used. Those individuals

involved in staining and interpretation were blinded to clinical information until the statistical analysis.

Statistical analysis

The clinical/pathological data and immunohistochemistry results were transferred into SAS (SAS Institute Inc, SAS/STAT User's Guide version 9.1, Cary NC SAS Institute Inc., 2003). Fisher's exact tests were used to compare demographic, clinical and pathological data, and trends were analyzed using the Jonckheere-Terpstra (JT) test. Since there were no cancer-related deaths in patients with LMP tumors, only EOC cases were used in these studies. In order to define a "high risk" category of EOC, we considered known factors implicated in EOC survival (age at diagnosis, race/ethnicity, tumor grade, histology, stage), and in our data only grade and stage were predictive. These variables were used to stratify patients into "high risk" EOC (all grade 3 and stage III/IV, grade 2) and "low risk" (all grade 1 and stage I/II, grade 2) groups. The LIFETEST procedure was used to calculate survival curves in EOC and in "high risk" EOC, and differences in survival were compared using the Log-rank test [26]. Two-tailed p values less than *0.05* were considered statistically significant.

Results

Table 1 depicts the distribution of patients with LMP tumors (45, 33.6%) and EOC (89, 66.4%) by race/ethnicity, age at diagnosis, histological subtype, tumor grade, and FIGO stage. Patients with LMP tumors were significantly younger (p = 0.003), had earlier (predominantly stage I) disease (*p < 0.001*), and the tumors were more frequently of mucinous histology (*p < 0.001*), in approximately 50% of the cases. The median H-score of 105 for ovarian cancer was used to divide patients into two groups: H-scores greater than 105 were defined as GPR30-High and H-scores up to 105 were defined as GPR30-Low..

Figure 1 illustrates representative staining from patients with LMP tumors. Benign cyst wall epithelial cells (Figure 1A, black arrow) and cytoplasmic vacuoles within mucinous tumors (Figure 1A, white arrow) were not immunoreactive. In many examples, intense staining of stromal tissue was observed, but was not included in the assessment of GPR30 expression. Serous LMP tumors typically displayed minimal cytoplasmic staining (Figure 1C,D,E); however, in 9/22 cases (40.9%), more intense epithelial cell staining was observed (Figure 1F). Nuclear staining was found in both LMP tumors and EOC (Figures 1E, 2C), and intense staining of inflammatory cells was observed within the extracellular matrix, (Figures 1B, 2B), but neither was used in the grading scheme.

In contrast to LMP and low-grade tumors, EOC typically exhibited greater epithelial staining (Figure 2). High grade, advanced stage neoplasms (Figure 2A,F) exhibited intense staining involving most of the epithelial cytoplasm, whereas well differentiated EOC (Figure 2B,D,E) had minimal epithelial staining. In eight paired samples, no significant differences in H scores were observed comparing C-terminal and N-terminal antibodies (Figure 2F).

Table 2 depicts GPR30 expression in EOC and LMP tumors stratified as either high or low by age at diagnosis, histological subtype, tumor grade, and FIGO stage. GPR30 expression above the median was observed more frequently in EOC than in LMP tumors [EOC, 43/89 (48.3%) vs. LMP tumors, 9/45 (20.0%), *p = 0.002*]. In EOC, the difference in GPR30 expression by grade overall (p = 0.07), and by grade 1 vs. 2 +3 EOC (p = 0.10) approached statistical significance. However, when LMP tumors were included in the analysis, the frequency of high GPR30 immunostaining increased with tumor grade (LMP tumors--20%, grade 1--30.1%, grade--2 40.9%, and grade 3--55.6%), a trend that was statistically significant (*p < 0.001*). When LMP and grade 1 tumors were stratified together and compared with high grade (2 +3) tumors, this difference was also significant (*p = 0.004*).

At the time of analysis, 91 patients were alive, 37 had died from EOC, and 6 had died from causes other than ovarian cancer. Other causes of death consisted of atherosclerotic cardiovascular disease (2), breast cancer (1), gallbladder cancer (1), and unknown causes (2); half of these affected patients with LMP (3) and half, EOC (3). In all of the survival analyses, deaths from other causes were censored. The median survival time was 49 months (range 0 to 140 months). All deaths from ovarian cancer occurred in patients with EOC. Since there were no ovarian cancer-related deaths in the patients diagnosed with LMP tumors, these were removed from the survival analyses. The 2-year, 5-year, and overall survival rates were 73.1%, 64.0%, and 58.4%, respectively.

Illustrated in Figure 3, in patients with EOC, overall (not shown), and 5-year survival rates were significantly influenced by tumor grade (Gr 1--100%, Gr 2--59.1%, Gr 3--57.4%, *p = 0.02*), and FIGO stage (Stage I--91.7%, Stage II--88.9%, Stage III--55.8%, Stage 4 23.1%, *p < 0.001*). By age at diagnosis (≤ 50 versus > 50 years), overall (77.8% vs. 50.0%, *p = 0.04*) but not the 5-year (77.8% vs. 58.1%, $p = 0.09$) survival was significant. However, there were no differences in overall (not shown) or 5-year survival rates by histological subtype (serous 64.7%; mucinous 46.7%; endometrioid 78.8%; and other cell types, 77.8%; p = 0.24), or by race/ethnicity (non-Hispanic white 70.7%, Hispanic 58.1%, American Indian 56.3%, p = 0.44). In subset analyses by tumor grade, 5-year survival rates were higher in patients with grade 1 than either grade 2 (p = 0.01) or grade 3 (p = 0.005) tumors, with no significant difference for grade 2 vs. 3 disease (p = 0.96).

Figure 4A depicts the differences 5-year survival by GPR30 expression. Overall (GPR30-Low 78.3% vs. GPR30-High 37.2%, p < 0.001), 2-year [GPR30-Low 89.1% vs. GPR30-High, 89.1% vs. 55.8%, p = 0.0004), and 5-year (GPR30-Low 82.6% vs. GPR30-High 44.2% vs. GPR30-Low, 82.6%, *p < 0.001*) survival rates all were significantly worse for patients with tumors that exhibited higher levels of GPR30 expression.

There were 22 patients in the "low-risk" category and 67 patients in the "high-risk group" (all grade 3 tumors and grade 2 stage III/IV disease), with respective 5-year survival rates of 100% and 52.2%. For the "high-risk" group (Figure 4B), the 5-year survival rates were significantly worse in those patients with tumors that exhibited higher GPR30 expression [GPR30-High 33.3% vs. GPR30-Low, 74.2 %, p = 0.001], as were the median survival times (48 months vs. 20.5 months).

Discussion

In this study, GPR30 expressed at higher levels was more frequently observed in EOC than in LMP tumors, and the frequency of cases with GPR30 expression above the median H score increased with increasing tumor grade and stage of disease. Expression of GPR30 above the median was associated with significantly lower survival rates. Using marginal survival methodology, 5-year survival was significantly influenced by GPR30 expression, FIGO stage, and tumor grade, but not by histological subtype or by race/ethnicity. These data are consistent with our previous report in endometrial [22] and breast [23] carcinoma, and taken together, support the conclusion that higher levels of GPR30 expression are linked to lower survival rates in hormonally responsive solid tumors including endometrium, breast, and ovary.

We demonstrated that GPR30 is a predictor of 5-year survival along with stage and grade. An analysis of survival by GPR30 expression in the "low-risk" group was not feasible, because there were no cancer-related deaths. A power analysis (80% power, α = 0.05), assuming that the 5-year survival rate for grade 1 EOC is 70% [4], determined that 180 patients with grade 1 tumors would be necessary in order to detect a 20% difference (80%

vs. 60%) in survival rates. Nevertheless, our finding that GPR30 is expressed at lower levels in grade 1 EOC and LMP tumors, which confer high survival rates, supports the hypothesis that the hormonal regulation of LMP tumors and grade 1 EOC are correlated, and are different from the hormonal regulation of high grade (2 +3) EOC.

It is generally accepted that estrogen exposure plays a causal role in the development of breast [27–29] and endometrial [30–32] carcinoma, and although less well studied, epidemiological and experimental data suggest that estrogen may play a role in ovarian carcinogenesis [16,33]. Women who develop EOC share many features in common with those who develop endometrial carcinoma including obesity [34,35], high dietary fat [36,37], low parity [38], and in both diseases, oral contraceptives are protective [39,40]. For both cancers, EGFR appears to be an independent predictor of response to chemotherapy and survival, especially in patients with high grade tumors [22,41,42]. In endometrial cancer, GPR30 expression inversely correlated with ER and PR expression, and correlated positively with EGFR expression [22], which supports the hypothesis that in high risk endometrial carcinoma, GPR30 is an alternative receptor for estrogen activity in tumors where ER is downregulated. Additional studies including both receptors are needed to determine if the relationship between GPR30 and ER expression in "high risk" ovarian cancer parallels our observations in "high risk" endometrial cancer. Although it is possible that systemic estrogen may not play a significant role in ovarian carcinogenesis, locally produced estrogen within the cell may influence ovarian carcinogenesis through ERα/β, and/ or GPR30 in an autocrine or paracrine fashion.

In EOC, ER positivity is present in about 60% of tumors [14–16]. However, the relevance of ER status alone or in association with PR as a prognostic indicator for response to therapy and survival is inconclusive, as most clinical studies evaluating response to chemotherapy or estrogen receptor modulators (SERMS) have not included receptor expression data in their analyses [13,15,43]. Tamoxifen has only a modest degree of effectiveness in ovarian cancer refractory to cytotoxic chemotherapy, with an objective response rate in all trials of only 11%, and the response rates reported vary widely [44,45]. New data indicate that in heavily pretreated ER-positive platin-resistant ovarian cancer patients, the aromatase inhibitor letrozole has some clinical activity with minimal adverse effects [46]. Studies that evaluate GPR30 expression in relation to the classical steroid receptors (ERα/β, PR) and response to chemotherapy are needed to elucidate the value of GPR30 as a prognostic indicator. Since many G protein-coupled receptors (including GPR30) induce EGFR phosphorylation, the inter-receptor cross-talk demonstrated by this paradigm represents a novel opportunity for therapeutic intervention [21,47].

Our data provides additional support of the "two-tiered" grading system for EOC [4,48,49]. We included histological types other than serous carcinoma, an approach supported by recent studies indicating that EOC can be subdivided into Type I, or "good prognosis" EOC, which includes low-grade micropapillary serous, mucinous, endometrioid, and clear cell carcinomas, and Type II tumors, which include high grade serous carcinoma, carcinosarcomas, and undifferentiated carcinomas [3]. Our observations indicate that the differences observed in GPR30 expression represent another important difference in the biology of Type I and II EOC.

The relationship between estrogen signaling and its multiple regulatory interactions with growth factor and other kinase signaling pathways involves complex patterns of genomic and non genomic cross-talk. Estrogen, as well as many of the classic ER antagonists and SERMs (including fulvestrant and tamoxifen) activate signaling pathways via GPR30 [18,50,51]. Therefore, we postulate that regulating the expression or function of GPR30 with selective agonists [52] and/or antagonists [53] may prove to be an effective treatment

strategy, in conjunction with standard chemotherapy. This hypothesis is further supported by the preclinical observation that G-1 (a selective GPR30 agonist) induces proliferation in ovarian carcinoma cell lines [54] and G15, a new selective GPR30 antagonist, inhibits estrogen-induced proliferation of uterine epithelium in a mouse model [53].

There are limitations to the current study. The impact of GPR30 on survival for "low risk" disease was not feasible because of the sample size. We did not observe a difference in OS by histological subtype, a known predictor of survival in EOC [55], probably also a reflection of the sample size. This is a retrospective study that utilized a centralized database instead of a prospective study. Despite these limitations, our data link GPR30 with many of the clinical and pathological features associated with poor prognosis in EOC and, as we observed for endometrial carcinoma [22], lower survival rates. Although the pathways involved in GPR30 signaling are complex and overlap with other mechanisms responsible for cell proliferation, elucidating the interactions involved may identify new opportunities for therapeutic intervention.

Acknowledgments

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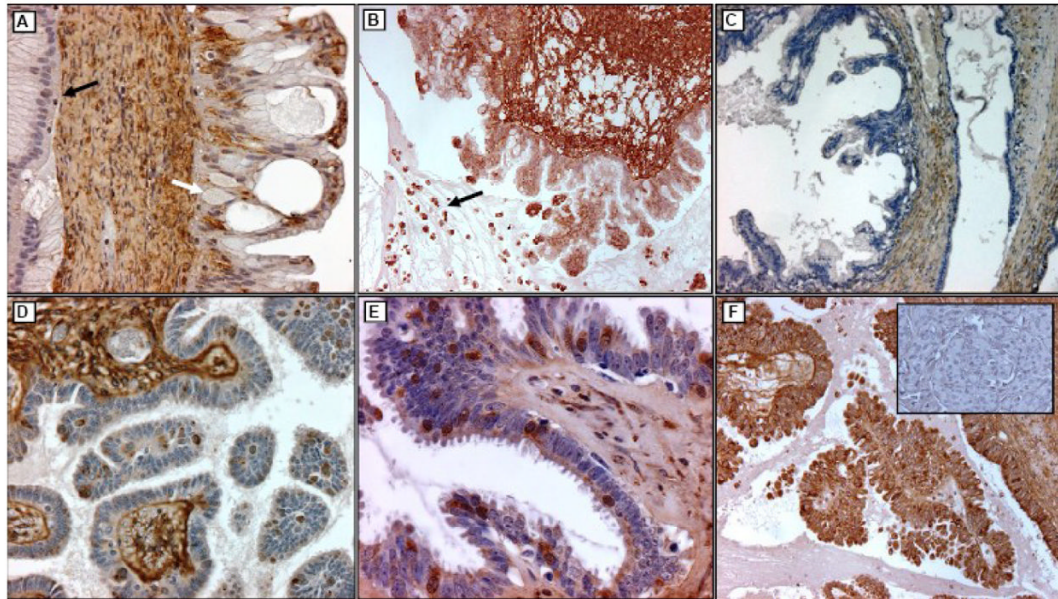
Figure 1.
Immunostaining for GPR30 in LMP tumors. **A)** FIGO Stage IA mucinous tumor; benign cyst wall (left, arrow) with no detectable staining; borderline tumor to right, with 2+ staining in 20% of epithelial cells; vacuoles do not stain (63X, oil); **B)** FIGO stage 1A mucinous tumor; intensity 1.5+ in 15% of epithelial cells and 3+ staining within 100% of stroma cells and within inflammatory cells (arrow), (40X); **C)** FIGO stage IA tumor; no epithelial cell staining, trace stromal staining (20X); **D)** FIGO Stage IC serous tumor; minimal epithelial cell and intense (3+) stromal staining (40X); **E)** FIGO Stage IIIB serous LMP tumor; intensity 1.5+ in 15% of epithelial cells; 3+ positive stromal staining (63X, oil). Nuclear/ nuclear envelope staining was also observed in 20% of epithelial cells; **F)** FIGO Stage IIC serous tumor; 3+ intensity in 100% of epithelial and stromal cells (20X). This was the most strongly positive example of all LMP tumors evaluated; inset (40X), negative control using isotype antibody and endometrial carcinoma tissue previously shown to be strongly positive. In this figure, all samples were stained with the C-terminal antibody.
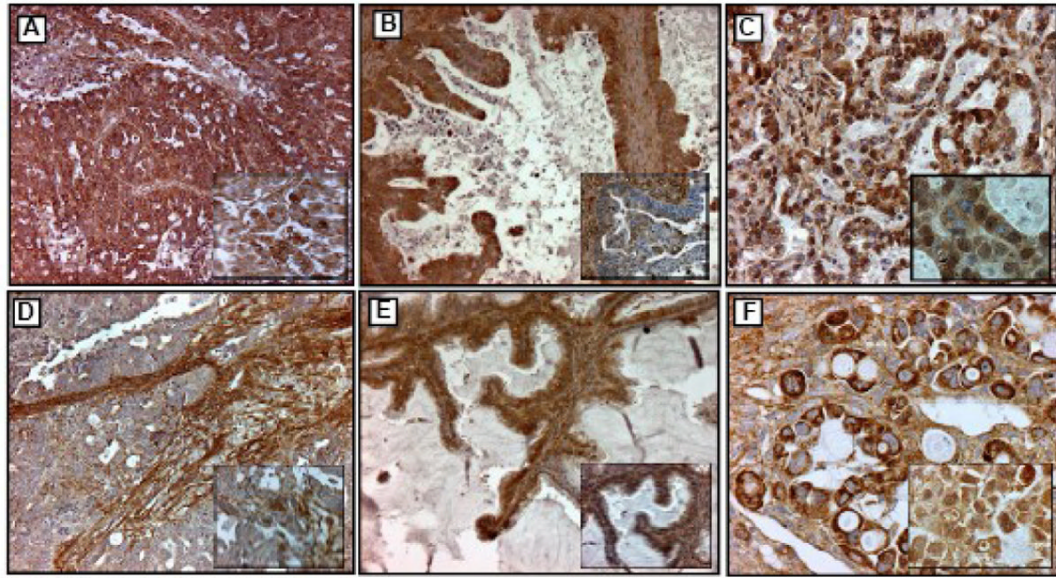
Figure 2.
GPR30 expression in ovarian carcinoma. **A)** FIGO Stage IIIC, Gr 3, serous carcinoma; status: post optimal cytoreduction and combination chemotherapy, dead of disease 8 months after treatment; intensity 3+ in 100% of epithelial cells (40X, inset 63X oil); **B)** FIGO Stage IA Gr 1 mixed serous and endometrioid carcinoma, disease-free at 63 months; intensity 1.5+ in 70% of epithelial cells (20X, inset 40X); **C)** FIGO Stage IA Gr 2 serous carcinoma; status: post 6 cycles, without evidence of recurrence at 140 months (40X); intensity 3+ in 30% of epithelial cells; in this example nuclear staining was present in 90% of epithelial cells, and predominated over cytoplasmic staining (inset, 63X, oil); **D)** FIGO Stage IIIC Gr 2 serous carcinoma, without evidence of disease at 108 months (40X). Although stromal staining was intense (3+), epithelial staining was minimal (2+ positive in 25% of epithelial cells, inset, 63X oil) with occasional nuclear staining); **E)** FIGO Stage IB Gr 3 mucinous carcinoma, disease-free at 60 months; 1.5+ intensity in 30% of cells (20X, inset 40X); **F)** FIGO Stage IIIC mucinous adenocarcinoma, dead of disease 2 months after diagnosis; 3+ intensity in 95% of cells (inset, N-terminal antibody, both images 63X). All samples, unless noted, were stained with the C-terminal antibody.
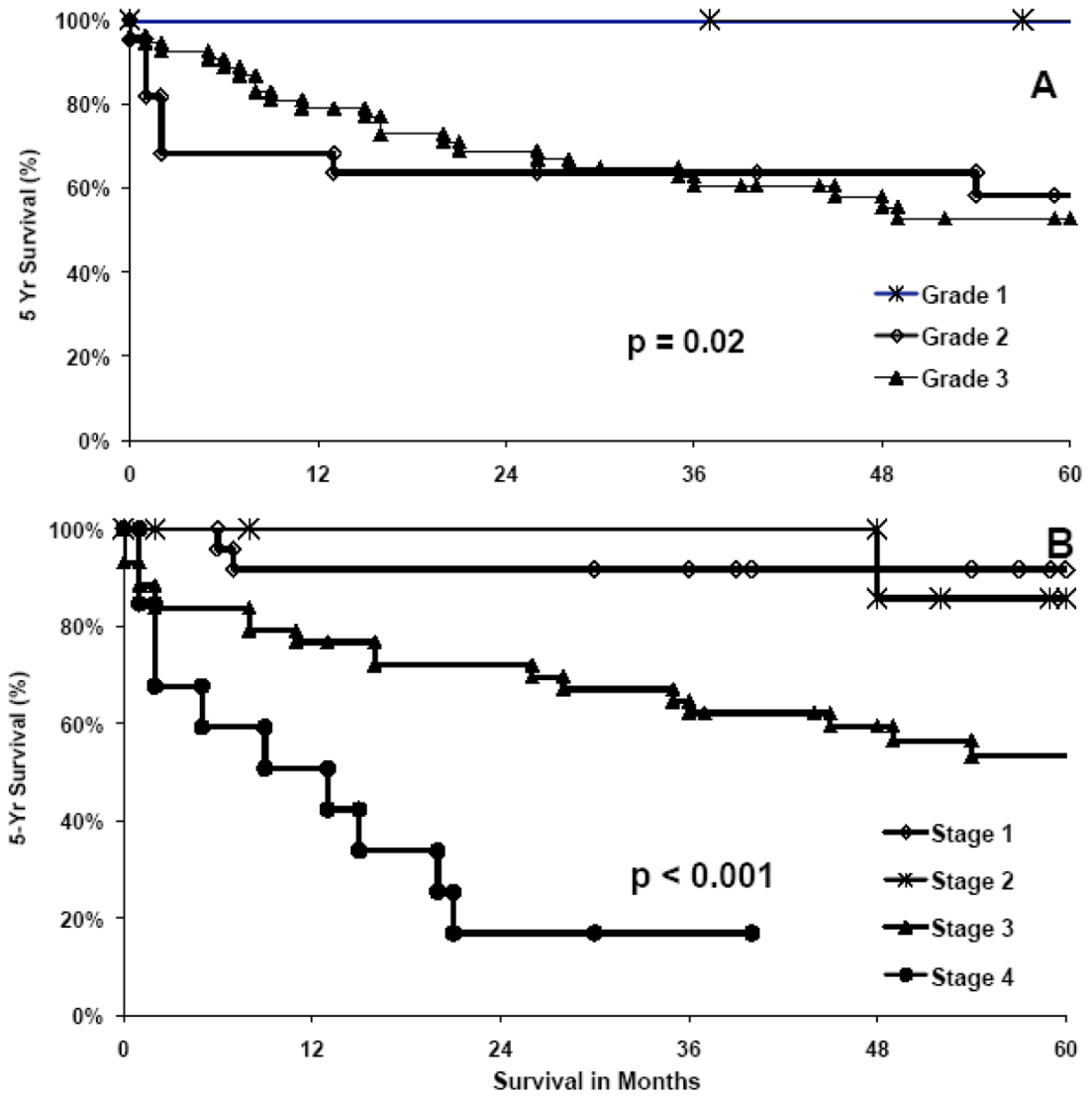
Figure 3.
Factors Significantly Impacting Survival Rates in Patients with EOC (N = 89). Five-year survival by (A) tumor grade; and (B) FIGO stage.
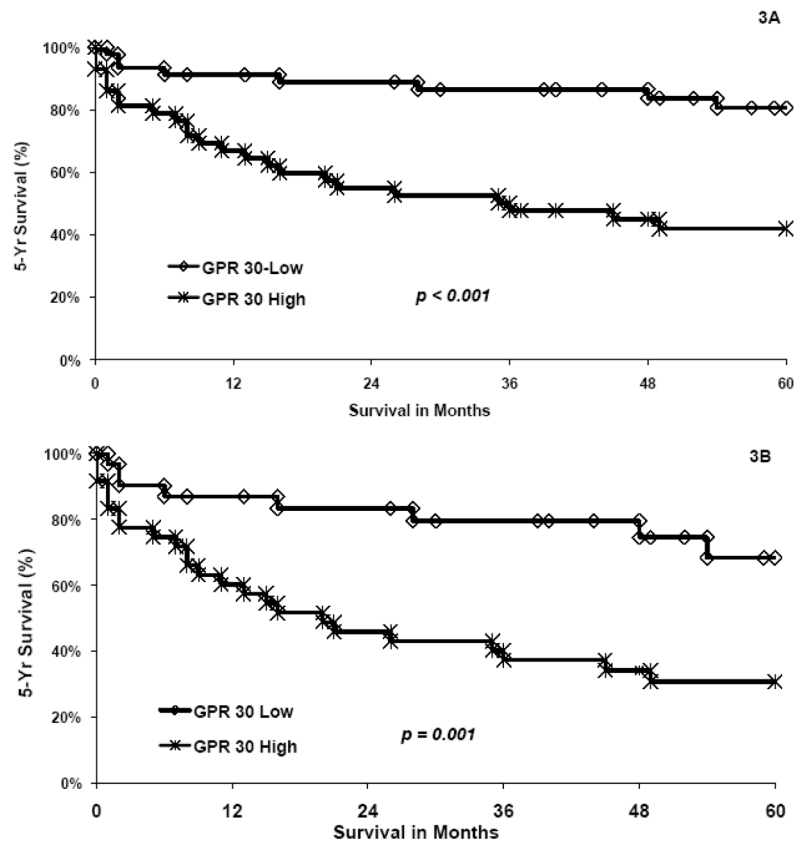
Figure 4.
Impact of GPR30 Expression on Survival Rates
Five-year survival in (A) all patients with EOC (N = 89); and (B) high-risk EOC (N = 67).

Table 1

Characteristics of LMP and Epithelial Ovarian Cancer Cases.

Variable	LMP (45) N (%)	EOC (89) N (%)	Total (134) N (%)	Fisher P
Race/Ethnicity				0.5
Non-Hispanic White	23 (51.1)	41 (30.6)	64 (47.7)	
Hispanic	15 (33.3)	31 (34.8)	46 (34.3)	
American Indian	5 (11.1)	16 (11.9)	21 (15.7)	
Other	2 (4.4)	0	2 (1.5)	
Age				*0.003*
≤ 50 yrs	26 (57.8)	27 (30.3))	53 (39.6)	
>50 yrs	19 (42.2)	62 (69.7)	81 (60.4)	
Histology				*< 0.001*
Serous	22 (48.9)	51 (57.3)	73 (54.5)	
Mucinous	22 (48.9)	15 (16.9)	37 (27.6)	
Endometrioid	1 (2.2)	14 (15.7)	15 (11.2)	
Other	0	9 (10.1)	9 (6.7)	
Grade				*< 0.001*
0	45 (100)	0	45 (33.6)	
1	NA	13 (14.6)	3 (9.7)	
2	NA	22 (24.7)	22 (16.4)	
3	NA	54 (60.7)	54. (40.3)	
FIGO Stage				*< 0.001*
I	40 (88.9)	24 (27.0)	64 (47.8)	
II	3 (6.7)	9 (10.1)	12 (9.0)	
III	2 (4.4)	43 (48.3)	45 (33.6)	
IV	0	13 (14.6)	13 (9.7)	
Total	**45 (33.6)**	**89 (66.4)**	**134 (100)**	

LMP, epithelial ovarian tumors of low malignant potential; EOC, epithelial ovarian cancer; Race/Ethnicity as defined by SEER coding; Other included all cases coded by race/ethnicity as other than the three predominant race/ethnic groups specified..

Table 2

Predictors of GPR30 Expression in LMP Tumors and Epithelial Ovarian Cancer (EOC).

Variable	LMP tumors (45)			EOC (89)			Totals (134)		
	GPR30 High N (%)	GPR30 Low N (%)	Fisher P	GPR30 High N (%)	GPR30 Low N (%)	Fisher P	GPR30 High N (%)	GPR30 Low N (%)	Fisher P
Age			0.46			0.18			0.02
≤ 50 yrs	4 (15.4)	22 (84.6)		10 (37.0)	17 (63.0)		14 (26.4)	39 (73.6)	
>50 yrs	5 (26.3)	14 (73.7)		33 (53.2)	29 (46.8)		38 (46.9)	43 (53.1)	
Histology			0.46[*]			0.58			0.20
Serous	6 (27.3)	16 (72.7)		22 (43.1)	29 (56.9)		28 (38.4)	45 (61.6)	
Mucinous	3 (13.6)	19 (86.4)		8 (53.3)	7 (46.7)		11 (29.7)	26 (70.3)	
Endometrioid	0	1 (100.)		7 (50.0)	7 (50.0)		7 (46.7)	8 (53.3)	
Other	0	0		6 (66.7)	3 (33.3)		6 (66.7)	3 (33.3)	
Tumor Grade			NA			0.08			0.003
0	9 (20.0)	36 (80.0)		NA	NA		9 (20.0)	36 (80.0)	
1				4 (30.8)	9 (69.2)		4 (30.8)	9 (69.2)	
2				9 (40.9)	13 (59.1)		9 (40.9)	13 (59.1)	
3				30 (55.6)	24 (44.4)		30 (55.6)	24 (44.4)	
FIGO Stage			0.26[**]			0.01			< 0.001
I	7 (17.5)	33 (82.5)		7 (29.2)	17 (70.8)		14 (21.9)	50 (78.1)	
II	2 (66.7)	1 (33.3)		2 (22.2)	7 (77.8)		4 (33.3)	8 (66.7)	
III	0	2 (100.)		24 (55.8)	19 (44.2)		24 (53.3)	21 (46.7)	
IV	0	0		10 (76.9)	3 (23.1)		10 (76.9)	3 (23.1)	

GPR30 High, expression above the median (80).

[*] In order to compute differences in LMP tumors, rows with 0 values were deleted and the single endometrioid was included with serous for analysis by histology.

[**] LMP Stage I (40 cases) was compared with LMP II + III (5 cases) in the analysis by stage of disease; NA, not applicable.