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17 β hydroxysteroid dehydrogenase type 12 (HSD17B12) is a marker of poor prognosis in ovarian carcinoma

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Abstract

Objective—17 β -hydroxysteroid dehydrogenase isoform 12 (HSD17B12) overexpression is associated with poor clinical outcome in invasive ductal carcinoma of the breast. Here, we evaluated HSD17B12 overexpression and its activity in ovarian carcinoma (OvCa) to determine its role in growth and progression of this tumor.

Methods—Immunohistochemical analysis of HSD17B12 expression was performed in 100 tissue samples of untreated OvCa and was correlated with clinicopathologic characteristics and patient outcome. In A2780 OvCa cell line expressing HSD17B12, siRNA knockdown of the enzyme was performed, and its effects on tumor cell growth and Annexin V binding were determined.

Results—HSD17B12 expression was detected in all tumor samples, but the staining intensity was variable. Normal ovarian epithelium was negative. Patients with tumor showing weak/moderate expression of HSD17B12 had a better overall survival than those with strongly positive tumors ($p < 0.001$). The time to first recurrence was longer for patients with tumors with heterogenous staining relative to patients with tumors that were uniformly positive ($p < 0.001$). Upon silencing of HSD17B12 in tumor cells, their growth was inhibited ($p < 0.005$) and apoptosis was increased ($p < 0.05$). Arachidonic acid but not estradiol reversed the growth inhibition mediated by HSD17B12 knockdown.

Conclusion—HSD17B12 overexpression is shown to be a marker of poor survival in patients with OvCa. Expression in the tumor and function of this enzyme facilitates OvCa progression.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

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Keywords

HSD17B12; ovarian carcinoma; prognosis; HSD17B12 silencing

INTRODUCTION

Epithelial ovarian cancer is a significant cause of death in the world. Most of ovarian cancer (OvCa) patients present with advanced disease at the time of initial diagnosis. Treatment for stage III and IV disease is rarely curative; five year survival rates are under 20–30% and have remained relatively constant for the past 30 years [1, 2]. Presumably the majority of OvCa are derived from ovarian surface epithelial inclusion cysts that undergo malignant transformation and differentiate towards various Müllerian cell types [3]. The role of biochemical, molecular and hormonal factors involved in carcinogenesis and pathogenesis of OvCa is not well understood. A growing body of evidence suggests that reproductive hormones can affect progression, proliferation and metastasis through a paracrine, autocrine or intracrine mechanisms [4, 5].

17 β -hydroxysteroid dehydrogenase (HSD17B) isoforms play an important role in the formation, inactivation and regulation of steroid hormones, such as estrogens and androgens. The enzymatic activities associated with different HSD17B isoforms are widespread in human tissues, including ovary. To date, fifteen isozymes of HSD17B have been reported [6, 7], each with a selective substrate affinity, directional (reductive or oxidative) cellular activity and a particular tissue distribution. HSD17B1, 7 and 12 are reductive estrogenic enzymes that catalyze the conversion of estrone (E1) to the more biologically potent estradiol (E2) [8]. In contrast, HSD17B 2, 4, 8, 10 and 14 are oxidative enzymes that are responsible for E2 inactivation [9, 10].

There is growing evidence for the role of HSD17B in the pathogenesis and development of various hormone-dependent carcinomas [11]. In the normal ovary, HSD17B is detected in granulosa cells of developing follicles, including primary, preantral and antral follicles, but not in the surface epithelium [12]. In contrast, a variety of epithelial OvCa have been reported to be positive for HSD17B [12, 13]. HSD17B12 is also overexpressed in other types of human carcinomas including human breast carcinoma (BrCa) and squamous cell carcinoma of the head and neck (SCCHN) [14, 15]. Nagasaki et al recently reported that HSD17B12 expression in 48% of total 110 invasive ductal BrCa was significantly associated with poor clinical outcome [14]. However, its potential role in tumor progression in BrCa as related to E1/E2 conversion is controversial [16]. The estrogens are likely involved in the genesis and progression of epithelial OvCa [17, 18] by promoting angiogenesis [19] and increasing secretion of pro-inflammatory cytokines, IL-6 and IL-8 [20]. Perhaps more critical to the ontogeny and progression of OvCa, however, is the ability of HSD17B12 to catalyze the elongation of long chain fatty acids, such as palmitic acid to arachidonic acid (AA) [21]. The latter is converted by cyclooxygenase-2 (COX-2) to prostaglandin E2 (PGE₂), an important mediator of inflammation. To determine whether HSD17B12 is also a critical clinicopathologic parameter in various epithelial OvCa, we analyzed its expression by immunohistochemistry (IHC) in 100 tumor specimens of untreated OvCa and correlated the results with clinical outcome. Using HSD17B12 knockdown by small-interfering RNA (siRNA), its effects on tumor cell proliferation and apoptosis as well as its primary metabolic role in OvCa were evaluated.

MATERIALS AND METHODS

Tumor cell lines

The human OvCa cell lines A2780 was established from tissue obtained from an untreated patient. These cells grow as a monolayer or can be cultured in suspension in spinner flasks. The AD10 cell line is adriamycin-resistant subline derived from the A2780 cell line, and it expresses a multi drug-resistant phenotype. These lines were provided by Dr. S Khleif, NIH, Bethesda, MD and were cultured in RPMI 1640 medium supplemented with 10% (v/v) FCS, 2mm L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in an atmosphere of 5%CO₂ in air. Tumor cell lines were tested for *Mycoplasma* and confirmed to be negative.

Tissue samples

Tissue samples were collected from 100 previously untreated patients with epithelial OvCa who had cytoreduction in the Gynecologic Oncology Clinic at the University of Medical Sciences in Poznan, Poland between years 1999 and 2005. The study was approved by the Ethics Committee of the University of Medical Sciences in Poznan. Histologic diagnosis including tumor grade were determined by the WHO criteria and were confirmed by a second review of the original H&E tissue sections. Clinical stage was determined by using the International Federation of Gynecology and Obstetrics (FIGO) criteria (Table 1). Normal ovarian tissues were used as controls.

Antibodies and immunohistochemistry

Five micrometer paraffin sections of tumor tissues were cut, deparaffinized, re-hydrated and microwaved, while immersed in 0.01 M citrate buffer pH 8.0 for 15 min. To avoid nonspecific binding of the secondary antibody (Ab), tissue sections were incubated with a serum-free protein blocker for 45 min prior to the staining procedure. After washing with PBS, sections were incubated with either a polyclonal rabbit Ab specific for the HSD17B12₁₁₄₋₁₂₂ peptide at 5µg/mL or with Abs to estrogen receptor (ER; clone 1D5) or progesterone receptor (PR; clone PgR 626) both purchased from DakoCytomation, for 30 min at room temperature (RT) in a moisture chamber [22]. Next, slides were washed in 0.5% BSA and then incubated with secondary anti-rabbit Ab labeled with horseradish peroxidase (HRP) under the same conditions. The antigen-Ab complexes were visualized with 3, 3'-diaminobenzidine (DAB) solution (1mM DAB, 50mM Tris-HCl buffer, pH 7.6) for 10 min. Tissue sections were counterstained with Mayer's hematoxylin solution (Sigma, St. Louis MO) and covered with mounting medium. In control sections, the primary Ab was omitted or HSD17B12₁₁₄₋₁₂₂ Ab was first incubated with peptide used to immunize the rabbit (1:5 weight ratios) for 3h and then used for staining. In negative controls for ER and PR, primary Abs were omitted. Slides were evaluated in a light microscope (x 200 magnification). For digital image analysis, the software Adobe Photoshop version 7.0 was used. The slides were evaluated by three independent investigators (E.E, M.S. M.J.S) and scored as positive, heterogenous, or negative, when the percentage of stained tumor cells in each section was >75%, between 25% and 75%, and <25%, respectively. The level of staining intensity was recorded as none, weak, moderate or strong. Positivity of staining was independent on intensity, and each of these parameters was considered in relating HSD17B12 expression in the tumor to patient survival.

Immunostaining

Tumor cells were deposited on glass slides, fixed in 2% (w/v) paraformaldehyde in PBS for 15 min, permeabilized with 0.1% (w/v) Triton X in PBS, washed and blocked with 2% (w/v) bovine serum albumin (BSA; Sigma, St. Louis MO) in PBS for 4 min. Polyclonal rabbit Ab

capable of recognizing the HSD17B12₁₁₄₋₁₂₂ peptide was used as a primary reagent at 5 µg/mL for 30 min at RT in a wet chamber. FITC-labeled (1:200) donkey anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz CA) was used as a secondary Ab. To eliminate non-specific binding of the secondary Ab, slides were incubated with 10% normal donkey serum for 1h prior to incubation with the primary Ab at RT. Slides were then washed with PBS and incubated with the secondary Ab for 45 min at RT in the dark. In control samples, primary Abs were omitted or goat serum was used instead of primary Abs. Sections were mounted in a medium with DAPI (Vector Laboratories, Burlingame CA) to visualize cell nuclei. Slides were evaluated with the Olympus Provis (Olympus America Inc., Center Valley PA) fluorescence microscope under 400x mag. and Adobe Photoshop 7.0 was used for digital image analysis.

Tumor cell proliferation

A2780 cells were plated overnight in wells of six-well plates at the density of 2×10^5 cells per well. They were cultured in medium alone or in the presence of small interfering RNA (siRNA) specific for HSD17B12 or with negative control siRNA (see below). The reagents were purchased from Santa Cruz Biotechnology. The viability and numbers of tumor cells were determined by microscope counts in the presence of a trypan blue dye using tumor cells harvested after treatment with TripLE Select solution (Invitrogen, Carlsbad CA) on day 1, 2 and 3 of culture.

Annexin-V binding

A flow-based ANX-V assay was used to measure tumor cell apoptosis as previously described [23]. Briefly, tumor cells were treated with paclitaxel (2 µM), or cisplatin (10 µM) as previously described [24, 25] for 6h, washed in PBS, resuspended in ANX-V-binding buffer and stained with 1 µg/mL PE-conjugated ANX-V (Sigma) and 7-amino-actinomycin-D (7AAD) for 15 min on ice in the dark. Tumor cell apoptosis was evaluated by flow cytometry, as previously described [24].

Silencing of HSD17B12 using siRNA

HSD17B12 expression was temporarily silenced in A2780 OvCa using siRNA, as previously described [14]. Briefly, 2×10^5 tumor cells were seeded in six-well culture plates in 2 mL of antibiotic-free growth medium supplemented with FBS. Subconfluent cells were collected and re-suspended in medium containing transfection reagents and human HSD17B12 siRNA (Santa Cruz Biotechnology) at different concentrations (2–8 µL). Negative control siRNA had no homology to known human sequences. Transfection was performed as recommended by the siRNA manufacturer. Efficiency of the transfection process was assessed using fluorescein isothiocyanate-conjugated control siRNA (sc-36869) and specific siRNA (sc-37007) (Santa Cruz Biotechnology). Cultures with the transfection efficiency of >90% as assessed by fluorescence microscopy were used for further studies. The viability of transfected tumor cells was tested by trypan-blue exclusion. To test the effects of AA and E2 on reversing siRNA-induced growth inhibition, the supernatant was removed 24h after transfection, and serum-free RPMI-1640 medium added with or without 1 µM AA (MP Biomedicals, Solon, OH) (1:10 dilution of a 1:100 dilution in PBS of a 1 mM AA/DMSO stock solution) or 1 nM estradiol (Sigma) (1:10 dilution of a 1:100 dilution in PBS of a 1 µM E2/ethanol stock solution). After 48h incubation, cells were harvested for analysis. The effect of HSD17B12 knockdown on HSD17B12 mRNA expression was monitored by determining the number of viable tumor cells, and by quantitative reverse transcription-PCR (qRT/PCR) using primers designed in our laboratory; *forward primer*: TTGCTGTTGACTTTGCATCAG; *reverse primer*: TTCACTAAGATGCCGATTTCAG and *probe*: 5'-/56 FAM/TGATAAAATTAATAA CAGGCTTGGCTGGT/3BHQ-1/3'.

Statistical analysis

To evaluate potential association between study variables of interest, we used Fisher's Exact tests, Cochran-Armitage Trend tests or Jonckheere Terpstra tests where appropriate. Adjustments to p-values were made using the Bonferroni step-down procedure. Overall survival (OS) and time to first tumor recurrence (TTR) were estimated using the Kaplan-Meier method. We also looked at OS and TTR stratified according to the staining pattern and intensity. Log-rank tests were used to evaluate differences among the strata in OS or TTR. The paired Student's t test was used to evaluate differences between treated versus untreated pairs of cell lines. The *p* values of <0.05 were considered significant.

RESULTS

HSD17B12 expression in OvCa tissues and cell lines

H&E tumor sections were first examined to select representative sections for immunostaining (Figure 1A1). In negative controls, primary Abs were omitted (Figure 1A2) or tonsillar tissues were used (data not shown). BrCa tissue samples were used as a positive control (Figure 1A3). Normal ovarian epithelium did not express HSD17B12; however, there was a very weak expression of HSD17B12 in ovarian stromal cells (Figure 1A4). All epithelial OvCa expressed HSD17B12 in the cytoplasm, with staining intensity varying from focal and weak (Figure 1A5) to diffuse and strong (Figure 1A6). Interestingly, 45 tumors (45%) demonstrated variability in staining intensity ranging from weak to strong within the tumor section (Figure 1B1–B3). This heterogeneous staining pattern was not associated with any specific histologic subtype of OvCa.

Smears prepared with A2780 and AD10 human OvCa cell lines were also examined for expression of HSD17B12 by immunofluorescence. A2780 showed a stronger staining (Figure 1C2) than AD10 cell line (Figure 1C3). No staining was observed when IgG1 was used as a negative control (Figure 1C1). A2780 cell line was chosen for further *in vitro* studies.

HSD17B12 expression correlates with clinical outcome in OvCa

Table 1 summarizes clinicopathologic characteristics of the OvCa patients enrolled in the study: menopausal status, stage, grade and histopathology subtype of tumor; surgical debulking; and recurrence. Patients ranged in age from 31 to 79 years, with the median age of 50 years. Median follow-up time for OS was 4.7 years (range=0 to 8.23 years). Median follow-up time for TTR was 5.05 years (range=5.8 months to 8.2 years). None of the covariates examined had an association with positivity or intensity, except for the FIGO stage which appears to have an association with staining positivity (at $p=0.010$, Table 2). There was a trend for increased proportions of positive staining with increasing FIGO stage scores (S. Table 1). The OS data were available for all 100 patients. Median OS was 3.14 years (95% CI: 2.29 to 4.19 years) (Figure 2A).

Patients with a heterogeneous staining pattern had better OS than patients with uniformly positive staining ($p<0.001$, Figure 2B). Among various staining intensities, patients with tumor showing weak and moderate staining intensities did not differ significantly in terms of OS ($p=0.157$). However, patients with tumors which had weak or moderate staining intensity had a better OS than patients whose tumors had a strong staining intensity ($p<0.001$, Figure 2C: Chi-sq, 2df, $p<0.001$). The TTR was calculated for 89 patients. Nine patients had no remission (progressive disease), one patient's recurrence date was unknown, and for one patient the data were not available. Median TTR was 1.51 years (95% CI: 1.33, 1.76 years) (Figure 2D). Patients with tumors that had heterogeneous staining also had a longer TTR relative to patients with tumors which were uniformly positive ($p=0.001$, Figure 2E). Considering staining intensities, TTR did not differ between patients with tumor

characterized by weak and moderate staining intensities ($p=0.177$). However, patients with tumors demonstrating strong staining intensity had shorter TTR than patients with weakly or moderately staining tumors ($p=0.004$, $p<0.001$, Figure 2F: Chi-sq, 2df, $p<0.001$). There was no correlation between ER or PR expression on tumor cells and the clinical outcome of the OvCa patients (data not shown).

siRNA-mediated inhibition of HSD17B12 expression in tumor cells

The expression of HSD17B12 in the A2780 OvCa cells was temporarily silenced using HSD17B12 specific siRNA. The transfection frequency was determined in a fluorescence microscope and was considered satisfactory when at least 90% of siRNA-FITC cells were positive (Figure 3A, *right panel*). The transfected cells were assessed for the successful silencing of HSD17B12 using real-time PCR. The optimal concentration of 4 μ L HSD17B12 siRNA was determined experimentally. Tumor cells temporarily silenced by siRNA decreased the expression of HSD17B12 mRNA level, as tested by real-time PCR (Figure 3A, *left panel*) on day 1, 2 and 3.

siRNA-mediated knockdown of HSD17B12 in A2780 cells inhibited cell growth and induced apoptosis

Tumor cells (2×10^5 cells per well) were cultured in medium for 24, 48 and 72h and analyzed for the cell growth. As shown in Figure 3B, *left panel* the significant growth inhibition was detected in tumor cells transfected with HSD17B12-specific siRNA compared with those transfected with non-targeting siRNA ($p<0.005$). Tumor cells treated with HSD17B12-specific siRNA showed an altered morphology, with many cells undergoing apoptosis compared to control (Figure 3B, *right panel*). Concordant with decreased cell growth was increased apoptosis of transfected cells. In the control cultures, spontaneous apoptosis levels ranged from 4 to 6.9%. After transfection with control siRNA, apoptosis levels remained low (3.5–7.5%). In contrast, dramatic changes in spontaneous apoptosis were observed in tumor cells transfected with specific siRNA after 24, 48 and 72h of transfection ($p<0.05$). Drug-induced apoptosis of tumor cells transfected with siRNA was also studied. While cisplatin or paclitaxel alone induced apoptosis in A2780 cells after 6h incubation, no differences in sensitivity of tumor cells silenced with siRNA to drug-induced apoptosis were observed (data not shown). These data suggest that inhibition of HSD17B12 activity does not further sensitize cells to drug-induced apoptosis.

Functional role of HSD17B12 in the A2780 OvCa cell line

The role of HSD17B12 activity in E1 to E2 conversion and elongation of long-chain fatty acids and synthesis of AA in A2780 cells was investigated using the approach described by Nagasaki et al [14]. We determined that AA, but not E2, could reverse the growth inhibition of A2780 cells by HSD17B12 knockdown (Figure 4A, B). This result indicates that the metabolic role of HSD17B12 in OvCa is not multifunctional, but limited to fatty acid elongation.

DISCUSSION

The results of this retrospective IHC analysis of HSD17B12 expression in OvCa indicate that elevated expression of this enzyme is an independent prognostic marker of poor survival in patients with this malignancy. Patients with tumors characterized by weak to moderate levels of HSD17B12 expression had increased median TTR and better OS than patients whose tumors stained strongly for this protein. These findings are consistent with the data reported for HSD17B12 expression in BrCa [14].

The *in vitro* experiments involving HSD17B12 knockdown with siRNA in an OvCa cells showed that expression of this enzyme and its function support tumor cell growth. Its knockout in OvCa cells resulted in a significant growth inhibition and apoptosis of the cells. These results confirm the critical role of HSD17B12 in OvCa growth and progression. Although HSD17B12 isoform has the potential to convert E1 to E2 and plays a role in hormonal regulation in normal and malignant breast and ovarian tissues, a controversy exists as to its involvement in E1/E2 conversion in these tissues [16]. It has been reported that another enzymatic activity attributed to this multifunctional HSD17B isoform, namely, elongation of long-chain fatty acids, is critical to the ontogeny and progression of BrCa [14]. HSD17B12 can catalyze the elongation of palmitic acid to AA, the precursor via COX-2 activity of PGE₂. PGE₂ is acknowledged to be a potent inflammatory agent associated with such critical aspects of tumor formation and progression as angiogenesis, proliferation, immunosuppression, metastasis and apoptosis [26]. Elevated levels of HSD17B12 have been shown to correlate with COX-2 overexpression in BrCa [14]. In OvCa, it has been well established that COX-2 expression, similar to HSD17B12 expression, is associated with a poor prognosis [26]. Our results demonstrating that AA but not E2 can reverse *in vitro* the growth inhibition of OvCa cells suggest that the role of HSD17B12 in this tumor, as in breast carcinoma, is primarily restricted to fatty acid elongation. Interestingly, the results of a similar analysis of the metabolic role of HSD17B12 in SCCHN [27] indicate that it functions in E1/E2 conversion as well as fatty acid elongation in this type of carcinoma.

The overexpression of HSD17B12 in OvCa and its role in promoting tumor growth suggest that it could serve as a target for therapeutic interventions. In addition to the development of metabolic inhibitors of this HSD17B isoform [28], it might be possible to consider HSD17B12-based immunotherapy in the future. In this regard, HSD17B12 has been identified in our laboratories as a CD8⁺ T cell-defined tumor antigen [27]. As a tumor cell component which is critical for cell survival and which is immunogenic (i.e., able to induce HSD17B12-specific CD8⁺ T cells), HSD17B12 emerges as an attractive new candidate for use in cancer vaccines against carcinomas overexpressing this enzyme.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- HSD17B12 expression was studied in OvCa tissues and cell lines.
- Weak/moderate HSD17B12 expression correlated with better OS and longer TTR
- Silencing of HSD17B12 in tumor cells induced growth inhibition and apoptosis.

Figure 1. HSD17B12 expression in ovarian tumor tissues and cell lines

Panel A1: H+E staining; Panel A2: Negative control reaction in OvCa (primary Abs were omitted); Panel A3: Immunostaining for HSD17B12 in a BrCa specimen (positive control tissue); Panel A4: normal ovarian tissue adjacent to the tumor; Panel A5: OvCa tissue showing weak positive reaction; Panel A6: OvCa tissue showing strong positive reaction (mag. X200); Panels B: HSD17B12 staining intensity in sections of the same tumor showing strong (B1), moderate (B2) or weak (B3) positive reactions (mag. X 200); relationship of weak, moderate and strong intensity within one section “*” - strong positive, black arrow-moderate, thick black arrow-weak (B4) (mag. $\times 100$); Panels C: Immunostaining for HSD17B12 in OvCa cell lines (mag. $\times 200$): negative control (C1), A2780 cell line (C2) and AD10 cell line (C3).

Figure 2. HSD17B12 expression and clinical outcomes in patients enrolled in the study
 (A) Kaplan-Meier plot of overall survival in patients with OvCa; (B) analysis of staining pattern and overall survival; (C) analysis of staining intensity and overall survival; (D) Kaplan-Meier plot of median time to first recurrence; (E) analysis of staining pattern and time to first recurrence; (F) analysis of staining intensity and time to the first recurrence. (A) Overall survival; (B) staining heterogeneity and overall survival; (C) staining intensity and overall survival; (D) median time to the first recurrence; (E) staining heterogeneity and time to the first recurrence; (F) staining intensity and time to the first recurrence.

Figure 3. The biological effects of HSD17B12 silencing in tumor cells using specific siRNA
 Silencing of HSD17B12 was measured by qRT-PCR (**A, left panel**); siRNA transfection efficiency (**A, right panel**) in tumor cells; Tumor cell proliferation (**B, left and right panel**); Apoptosis of tumor cells silenced with specific siRNA (**C, left and right panel**).

Figure 4. Growth inhibition of A2780 OvCa cells by HSD17B12 siRNA

(A) HSD17B12 mRNA synthesis in A2780 cells was inhibited by HSD17B12 siRNA, even in the presence of AA or E2 compared with control siRNA. (B) HSD17B12 siRNA inhibited growth of A2780 cells, which was reversed in the presence of 1 μ M AA but not 1 nM E2.

Table 1

Clinicopathologic characteristics of OvCa patients whose tumor tissues were evaluated by immunohistochemistry.

		<u>%</u>
Positivity for HSD17B12:	Positive	55
	Heterogenous	45
Menopausal Status:	Pre-menopausal	48
	Post-menopausal	52
Staining Intensity:	Weak	24
	Moderate	47
	Strong	29
FIGO Stage:	I A,B,C	12
	II A, B, C	10
	III A, B, C	78
Diagnosis Grade 3:	Serous	48
	Clear cell	08
	Transitional	03
	Undifferentiated	04
Grade 1 and 2:	Endometroid	33
	Mucinous	04
Cytoreduction:	Optimal	30
	Non-optimal	70
Recurrence:	No recurrence	19
	Recurrence	71
	Unknown	01
	No remission	09
Number of Recurrences:	0	19
	1	49
	2	18
	3	02
	Unknown	03
	No remission	09
Vital status:	Alive	29
	Dead	71

Table 2

Associations of clinicopathologic data with POSITIVITY and INTENSITY of staining for HSD17B12 expression in OvCa tumor tissues.^a

	Raw P value	Adjusted P value
<u>POSITIVITY</u>		
Cytoreduction *	0.133	0.665
Diagnosis *	0.898	1.000
Memopausal Status *	0.422	1.000
FIGO Stage **	0.002	0.010
Grade **	0.406	1.000
Number of Recurrences (excluding patients with no remission, n=88) **	0.342	1.000
<u>INTENSITY</u>		
Cytoreduction *	0.066	0.397
Diagnosis *	0.297	1.000
Memopausal Status *	0.596	1.000
FIGO Stage ***	0.422	1.000
Grade ***	1.000	1.000
Number of Recurrences (excluding patients with no remission, n=88) ***	0.892	1.000

^aSignificant p-values are highlighted

* Fisher's Exact test;

** Cochran armitage Test;

*** Jonckheere Terpstra Test