Title Page

17β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 IMPROVES SURVIVAL IN SEROUS EPITHELIAL OVARIAN TUMORS

Enrique Pedernera
Universidad Nacional Autónoma de México, Facultad de Medicina, Departamento de Embriología y Genética, Ciudad de México. México.

Flavia Morales-Vásquez
Instituto Nacional de Cancerología, Ciudad de México. México.

María J. Gómora
Universidad Nacional Autónoma de México, Facultad de Medicina, Departamento de Embriología y Genética, Ciudad de México. México.

Miguel A. Almaraz
Universidad Nacional Autónoma de México, Facultad de Medicina, Departamento de Embriología y Genética, Ciudad de México. México.

Esteban Mena
Universidad Nacional Autónoma de México, Facultad de Medicina, Secretaría General, Ciudad de México. México.

Delia Pérez-Montiel
Instituto Nacional de Cancerología, Ciudad de México. México.

Elizabeth Rendon
Hospital Militar de Especialidades de la Mujer y Neonatología. Ciudad de México. México.

Horacio López-Basave
Instituto Nacional de Cancerología, Ciudad de México. México.

Juan Maldonado-Cubas
Universidad La Salle, Ciudad de México. México

Carmen Méndez
Universidad Nacional Autónoma de México, Facultad de Medicina, Departamento de Embriología y Genética, Ciudad de México. México. E-mail mendezmc@unam.mx
Short Title
HSD17B1 in Serous Epithelial Ovarian Tumors

Keywords
ovarian cancer, epithelial ovarian tumor, overall survival, 17β-hydroxysteroid dehydrogenase, aromatase, estrogen receptor

Word count
3137 words
Abstract

The incidence of ovarian cancer has been epidemiologically related to female reproductive events and hormone replacement therapy after menopause. This highlights the importance of evaluating the role of sexual steroid hormones in ovarian cancer by the expression of enzymes related to steroid hormone biosynthesis in the tumor cells. This study was aimed to evaluate the presence of 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1), aromatase and estrogen receptor alpha (ERα) in the tumor cells and their association with the overall survival in 111 patients diagnosed with primary ovary tumors. Positive immunoreactivity for HSD17B1 was observed in 74% of the tumors. In the same samples, aromatase and ERα revealed 66% and 47% positivity, respectively. No association was observed of HSD17B1 expression with the histological subtypes and clinical stages of the tumor. The overall survival of patients was improved in HSD17B1 positive group in Kaplan Meier analysis (P = 0.028), and HSD17B1 expression had a protective effect from multivariate proportional regression evaluation (HR=0.44; 95%CI 0.24 – 0.9; P = 0.040). The improved survival was observed in serous epithelial tumors but not in non-serous ovarian tumors. The expression of HSD17B1 in the cells of the serous epithelial ovarian tumors was associated with an improved overall survival. Whereas aromatase and ERα were not related to a better survival. The evaluation of hazard risk factors demonstrated that age and clinical stage showed worse prognosis, and HSD17B1 expression displayed a protective effect with a better survival outcome in patients of epithelial ovarian tumors.
Introduction

According to statistics, ovarian cancer is the eighth leading cause of death by neoplasia in women worldwide (1). The most frequent type of ovarian tumor is epithelial ovarian cancer (EOC) (2). The EOC is further divided into four histological types: serous, endometrioid, mucinous, and clear cells. Serous tumors are the most frequent and are classified as: borderline tumor (BT), low-grade serous carcinoma (LGSC), and high-grade serous carcinoma (HGSC). Endometrioid-type tumors are classified by grade and identified as borderline, well differentiated and poorly differentiated, while mucinous tumors are considered borderline and carcinoma (2).

The incidence of ovarian cancer has been epidemiologically related to female reproductive events. Further, ovarian tumors are considered sensitive to sex steroid hormones, as they are mediated by specific androgen, estrogen, and progesterone receptors (3). Additionally, the steroid hormone receptor shows a particular expression profile in each type of ovarian tumor (4).

Evidence proves the expression of enzymes in several types of tumors involved in the metabolism of steroid hormones. Moreover, their presence in breast and prostate cancer has been extensively explored at the tissue level (5-8). As for ovarian cancer, intratumorally production of steroid hormones has been suggested (9). While in ovarian carcinoma, the expression of steroids sulfatase (10), sulfotransferase (11), aromatase (12), 17β-hydroxysteroid dehydrogenase types 1, 2, 4, 5, 7, 8, 12 (13-16) has been identified, supporting the possibility of intracrine production of steroid hormone.

17β-hydroxysteroid dehydrogenase (HSD17B) enzymes are members of the short-chain dehydrogenase/reductases (SDR) superfamily (17) and are involved in the activation or inactivation of steroid hormones. HSD17B1 is the first identified member of the family, and its
main activity was the conversion of estrone (E1) to 17β-estradiol (E2) (18); in humans, this enzyme is mainly located in the ovary and placenta (19).

Evidence of HSD17B1 activity was found in the microsomal fraction of ovarian serous carcinoma by identifying the conversion of E1 to E2 (20). The presence of HSD17B1 has been described in 10 out of 58 cases of ovarian carcinoma, registering a lower frequency than HSD17B types 2, 4 and 8 (21). In addition, the presence of HSD17B1 has been demonstrated in OVCAR-3 and SKOV-3 cell lines, which are derived from ovarian carcinoma (21). These enzymes have been proposed to determine estrogen levels in the tumor cells, suggesting they are involved in estrogen-mediated tumor cell proliferation (13-15, 21). Additionally, the inactivation of DHT due to 17B-HSD1 activity by 3beta-reduction has been described in breast cancer (22). However, the presence of HSD17B1 in ovarian tumor cases, its role in ovarian tumor cell metabolism, and its prognostic significance in ovarian cancer patients should be further evaluated.

This study was aimed to evaluate the expression of HSD17B1 in ovarian tumor samples obtained from initial laparotomy performed for diagnostic purposes. The association of HSD17B1 with aromatase and estrogen receptor alpha (ERα) expression was also evaluated. HSD17B1 expression translated into an improved survival rate, being an independent prognostic factor of overall survival.

**Materials and Methods**

**Patients and tissue sample**

The present retrospective cohort study includes 111 patients diagnosed with primary epithelial ovarian tumor of Instituto Nacional de Cancerología at México City. The included cases spanned a decade from 2008 to 2018. None of the patients included received chemotherapy or radiotherapy.
prior to diagnosis. After cytoreductive surgery, patients were treated according to the standardized protocol of the hospital based on carboplatin and paclitaxel chemotherapy. Variation in the number of treatment cycles was not considered for the cohort integration. Each patient signed a written informed consent regarding their participation in the study. The study was approved by the Faculty of Medicine Ethics Committee of the Universidad Nacional Autónoma de México (FM/DI/114/2022) and the Instituto Nacional de Cancerología (019/060/OMI).

Tumor tissue samples obtained during the initial laparotomy were processed following the prescribed protocols of the hospital tumor bank for handling and were embedded in paraffin for further study.

**Immunohistochemistry**

Samples for immunohistochemistry were fixed in 4% buffered paraformaldehyde (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) for 24 h and embedded in paraffin. Slices of 3 µm were recovered on coated slides. Hydrogen peroxide 1:100 v/v was used to block endogenous peroxidase and equine serum to avoid nonspecific binding. Epitope recovery was obtained with Diva Decloaker citrate buffer (Biocare Medical, Pacheco, CA, USA) in a pressure cooker. The primary antibodies used were ERα (HC20), rabbit polyclonal diluted 1:100 (Santa Cruz Biotechnology, Inc.); antibody against aromatase (GTX32456), rabbit polyclonal diluted 1:200 (Gene Tex, Inc., Irvine, CA, USA); antibody against HSD17B1, rabbit polyclonal diluted 1:200 (Gene Tex, Inc., Irvine, CA, USA). The secondary antibody used was Mach2 anti-rabbit HRP detected with diaminobenzidine (DAB) chromogenic kit (Biocare Medical, Pacheco, CA, USA). For negative controls the primary antibody was suppressed. Microphotographs were obtained with objectives HCX PL Fluotar, and a camera Leica DPC 160C (Leica Microsystems, Wetzlar, Germany).

The positivity of the immune reaction was established according to the intensity and percentage of labeled cells. Intensity was assessed as: 1- light; 2- medium; 3- strong. The percentage of
labeling was recorded as: 1- 10-25%; 2- 26-50%; 3- 51-80%, and 4- more than 80% (23). The combination of intensity and percent labeling greater than 2 was considered a positive reaction. Validation of double-blind samples was performed by three independent observers (MJG, MAA, and EPA).

Patient evaluation

Overall survival was established based on the date of initial surgery with diagnosis of primary ovarian tumor classified as: borderline tumor (BT), low-grade serous carcinoma (LGSC), high-grade serous carcinoma (HGSC), endometrioid, mucinous, and clear cells; the first three were included in the serous tumor group, and endometrioid, mucinous, and clear cells were incorporated in the non-serous group. Tumor stage was classified along with the International Federation for Gynecology and Obstetrics (FIGO) stage of disease. Patient status was followed up to 2022. Information was collected from hospital patient records.

Statistical analysis

The description of the clinical characteristics of the patients was evaluated by comparison of proportions (Z-value). Analysis of the association between HSD17B1 expression, tumor subtypes and FIGO clinical stage was performed by Chi-square test. Significance in Kaplan-Meier survival curves was obtained using log-rank values. Hazard ratio and confidence intervals were obtained from the univariate and multivariate Cox proportional regression model. The data was processed with SSPS 21 software. Significance was present when “P” value was less than 0.05.

Results

Table 1 shows the characteristics of the patients who were included in the study and the percentage of patients with tumors positive and negative for HSD17B1 expression. The median age of the patient was 48 years, with a similar age concerning HSD17B1 expression. Borderline
tumors and HGSC were the most frequent and did not show a significant difference regarding HSD17B1 expression. A similar observation was recorded for endometrioid and mucinous tumors. In the case of clear cell carcinoma, all five tumors included in the group were positive for HSD17B1. Regarding the clinical stage, 44% of the patients were FIGO stage III and IV: 42% in the HSD17B1-positive group and 46% in the HSD17B1-negative group. Menopause was present in 52% of the cohort, with a non-significant difference between the HSD17B1 positive and negative groups. Almost 83% of the patients were successfully cytoreduced. Expression of aromatase and ERα display similar frequency in both groups of HSD17B1. Information regarding menopausal status and surgical cytoreduction was missing for some of the patients.

Immunohistochemistry for HSD17B1 showed a positive reaction in the epithelial tumor cell cytoplasm; a similar distribution for aromatase was observed in histological sections of the tumors. ERα is identified in the nucleus of the tumor cells and eventually in stroma cells (Figure 1).

Most tumors (74%) are positive for HSD17B1. The aromatase and ERα immunoreactivity evaluation in the same tumor samples revealed 66% and 47% of positivity, respectively. The association between the expression of the three proteins was assessed through the Chi-square test in cross-tabulations. No association was observed between HSD17B1 and aromatase, nor between HSD17B1 and ERα (data not shown).

Table 2 shows the association between HSD17B1, aromatase, and ERα with histological subtypes of tumors. No significant association was observed for any of the three variables. Evaluation of the association of HSD17B1, aromatase, and ERα with the clinical stage according to FIGO showed no association with clinical stage (Table 3).

Survival curves obtained from the cohort were evaluated using Kaplan-Meier analysis, and the results indicated that patients with HSD17B1-positive tumors had a better survival rate than
patients with HSD17B1-negative ones (Figure 2A). No significant differences in survival were observed in tumors with aromatase and ERα expression (Figure 2A). These observations were maintained in serous epithelial ovarian tumors (Figure 2B) and were not registered in non-serous ovarian tumors (Figure 2C). Additionally, stratifying the patients according to the aromatase and ERα positivity or negativity in the tumor, it was identified that the higher survival rate for the HSD17B1-positive group was maintained in the aromatase-positive selection improving the statistical significance despite the reduction in the number of cases (Figure 3A). In contrast, when selecting and evaluating aromatase-negative tumors, the ameliorative effect of HSD17B1 expression was not observed Figure 3A). A similar result was observed when stratifying patients according to the presence of the estrogen receptor. ERα-positive tumors maintained better survival of the HSD17B1-positive group; this effect disappeared in ERα negative expression selection (Figure 3B).

Table 4 shows the hazard ratio after Cox proportional regression analysis. Univariate analysis showed that age and clinical stages III and IV have a worse prognosis; in contrast, HSD17B1 had a protective effect (HR=0.44; 95%CI 0.24 – 0.9); while aromatase and ERα were not significant as risk factors. In multivariate analysis, HSD17B1 maintained HR significance considering clinical stage and age at diagnosis as covariates.

**Discussion**

The present study evaluates the expression of HSD17B1 in epithelial ovarian tumors and how this is associated with improved overall survival of patients. The cohort includes patients of the Instituto Nacional de Cancerología, a tertiary-level hospital located in Mexico City where patients are received mainly from the central region of Mexico. The patients have been followed for over a decade to establish overall survival. It is important to note that the characteristics of the cohort do
not necessarily represent the entire population of patients with epithelial ovarian tumors since the absence of previous chemotherapy is an inclusion criterion; consequently, patients in advanced clinical stages who received neoadjuvant therapy prior to initial surgery were excluded. This restriction would be the explanation for the results obtained when evaluating the entire cohort through Kaplan-Meier analysis, which showed a low decrease in survival over time.

The lack of association between the presence of HSD17B1 with the presence of aromatase and ERα suggests there is no common regulation in their expression. Moreover, the absent relationship between ovarian tumor histological subtypes and stage of invasion suggests a general expression of the mentioned proteins in tumoral cells. Previous studies have shown frequent expression of HSD17B (21), aromatase (12) and ERα (4) in epithelial ovarian tumors.

Patients with a negative expression of HSD17B1 in tumor epithelial cells show reduced survival compared with patients with a positive expression of HSD17B1; such survival is halved after ten years of follow-up. The protective effect of HSD17B1 was also evidenced through Cox proportional regression analysis. Moreover, patient survival shows a similar decrease over time, regardless of the presence or absence of aromatase and ERα. These observations indicate that HSD17B1 expression plays a key role in the possible significance of 17β-estradiol biosynthesis and its effect on overall survival. Interestingly, the arms of the HSD17B1 curve exhibit a similar pattern during the first two years of follow-up, gradually diverging in the long term, while remaining statistically significant throughout the study. The observation suggests that the protective effect of HSD17B1 is evident in less aggressive tumors. Furthermore, the stratification of the cohort into patients with aromatase-positive tumors resulted also in a better survival for HSD17B1 positive tumors. Something similar was observed when selecting ERα-positive tumors, suggesting that the improvement in overall survival would be related to 17β-estradiol effect. Interestingly, the tumors
that are negative for HSD17B1, ERα, and aromatase do not display changes in survival confirming the probable involvement of 17β-estradiol in the protective effect herein described.

The presence of aromatase and HSD17B1 is related to the production of active steroids, which were previously proposed to serve as a source of ligands for the estrogen receptor on tumoral cells in breast, ovarian and endometrial cancer (24). Aromatase activity is required for the biosynthesis of estrone (E1) and 17β-estradiol (E2). In the presence of aromatase alone, E1 production could be expected, whereas expression of HSD17B1 will allow the production of active E2 (18); thus, the intracellular E1/E2 ratio will vary depending on HSD17B1 activity. The presence of HSD17B1 together with aromatase and ERα indicates that the effect of 17β-estradiol will be favored in the tumor cell, and improved survival is observed. Improved overall survival has been previously observed in ovarian carcinoma, when the mRNA of HSD17B variants favoring the reductive pathway is highly expressed, resulting in E2 production (25). Moreover, plasma levels of E1 have been reported to be risk factors in ovarian and colon-rectal tumors (26, 27). We propose that if estrogen production is shifted to E2 and E1/E2 ratio is reduced, then the tumor microenvironment would have a protective effect.

Previous studies in ovarian cancer cell lines show an increase in tumor cell proliferation after E2 treatment (28). Moreover, hormone replacement therapy in menopausal women is recognized as a risk factor for ovarian cancer (29); consequently, tumor progression associated with the presence of E2 might be expected. Interestingly, a study based on molecular data of The Cancer Genome Atlas (TGCA) demonstrates that estrogen receptor is a significant node in genetic interaction networks of gynecological cancer including ovarian high grade serous carcinoma (30). Present observations apparently contradict previous results; however, the effect of estrogens on the total tumor growth and the survival has not yet been elucidated in epithelial ovarian tumors (31-33).
The current results demonstrate that HSD17B1 is associated with increased survival. Therefore, changes in the balance between E1 and E2 could provide an explanation for the observed results. Alternatively, the involvement of the progesterone receptor (PR) in tumor progression should also be considered; the receptor is regulated by E2, and clinical studies have shown it to be a protective factor (34). In addition, progesterone in vitro reduces cell number and increases apoptosis in ovarian cancer cells (35, 36). There is also a need to consider the expression of ERβ in tumor cells, as its presence has been proposed as tumor suppressor by reducing cell cycle-related proteins and inhibiting epithelial-to-mesenchymal transition in ovarian cancer cell lines (37, 38). Interestingly, the improved survival associated with HSD17B1 presence is observed in serous tumors compared to non-serous tumors. A probable explanation would be related to the number of dead events in the serous group against the non-serous tumors, 21/62 versus 7/49, respectively, and supported by variations in the genetic feature of tumor cells and the prognosis of patients (2). Further studies will be necessary to understand the mechanism involved in the improved survival of patients with the HSD17B1 expression in the tumor cells.

A limitation of the present study is the number of tumors which are evaluated; consequently, the influence of confounding factors could be underestimated. However, the hazard ratio for HSD17B1 positive expression is independent of age and FIGO stage, two variables that affect overall survival (39). Moreover, the present results suggest that estrogens are involved in the progression of epithelial ovarian tumors and favor better patient overall survival in serous epithelial ovarian tumors. These facts do not necessarily occur in the same sense as in breast and endometrial cancer, opening new questions regarding the pathogenesis of ovarian cancer.

Conclusions
The expression of HSD17B1 in the cells of serous epithelial ovarian tumors is associated
with an improved overall survival of the patient. The evaluation of hazard risk factors
demonstrates that HSD17B1 displays a protective effect with better survival outcome
independent of the age and the FIGO stage of patients.

Declaration of interest

The authors declare that they have no conflicts of interests.

Funding

The present work was supported by a grant from DGAPA-PAPIIT, IN223823
to CM; a grant from DGAPA-PAPIIT, IN208822 to EP; MAA received a CONACYNT
scholarship.

Acknowledgements

We are deeply grateful to Mrs. Angélica Caballero and MC Isis Santos-Paniagua for their technical
assistance.

References

2021 71 7-33. (https://doi.org/10.3322/caac.21654)

2- Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and

(https://doi.org/10.1007/s00428-012-1203-5)

3- Shafrir AL, Rice MS, Gupta M, Terry KL, Rosner BA, Tamimi RM, Hecht JL, Tworoger SS. The
association between reproductive and hormonal factors and ovarian cancer by estrogen-α and


6- Hilborn E., Stål O., Jansson A. Estrogen and androgen-converting enzymes 17β-hydroxysteroid dehydrogenase and their involvement in cancer: with a special focus on 17β-hydroxysteroid dehydrogenase type 1, 2, and breast cancer. *Oncotarget* 2017 **8** 30552-30562. (https://doi.org/10.18632/oncotarget.15547)


22- Aka JA, Mazumdar M, Chen CQ, Poirier D, Lin SX. 17beta-hydroxysteroid dehydrogenase type 1 stimulates breast cancer by dihydrotestosterone inactivation in addition to estradiol production. *Molecular Endocrinology* 2010 **24** 832-845. (https://doi.org/10.1210/me.2009-0468)


33- Høgdall EV, Christensen L, Høgdall CK, Blaakaer J, Gayther S, Jacobs IJ, Christensen IJ, Kjaer SK. Prognostic value of estrogen receptor and progesterone receptor tumor expression in Danish ovarian cancer patients: from the 'MALOVA' ovarian cancer study. *Oncology Reports* 2007 **18** 1051-1059. (https://doi.org/10.3892/or.18.5.1051)


suppressor of epithelial ovarian cancer. *PLoS One* 2012 7 e44787

(https://doi.org/10.1371/journal.pone.0044787)


(https://doi.org/10.3390/cancers14092311)


(https://doi.org/10.1186/s13048-021-00840-x)

**LEGENDS TO FIGURES**

Figure 1.

Immunohistochemistry for HSD17B1, aromatase and ERα in a-c) High grade serous carcinoma; d-f) Endometrioid carcinoma; g-i) Mucinous carcinoma; j-l) Serous borderline tumor.

Photomicrographs were obtained from similar regions of triple-positive samples. HSD17B1 and aromatase reactivity are detected in the cytoplasm of epithelial cells, ERα is visualized in a nuclear location. Bars represent 50 µm.
Figure 2.

Survival curves of patients with epithelial ovarian tumor after Kaplan Meier analysis according to HSD17B1, ERα, and aromatase positive and negative expression. A) Whole cohort (n= 111), HSD17B1 positive n= 83, negative n= 28; Aromatase positive n= 72, negative n= 38; ERα positive n=51, negative n=59. B) Serous epithelial ovarian tumors (n= 62), HSD17B1 positive n= 45, negative n= 16; Aromatase positive n= 39, negative n= 22; ERα positive n= 30, negative n= 31. C) Non-serous ovarian tumors (n= 49), HSD17B1 positive n= 37, negative n= 12; Aromatase positive n= 33, negative n= 16; ERα positive n= 21, negative n= 28. P values were obtained from log-rank test.

Figure 3.

Survival curves for HSD17B1 in the whole cohort of epithelial ovarian cancer stratified according to aromatase and ERα expression. A) Aromatase positive tumors n=72, HSD17B1 positive arm n=54, negative arm n=18; aromatase negative tumors, n= 38 HSD17B1 positive arm n= 28, negative arm n= 10; B) ERα positive tumors n= 51, HSD17B1 positive arm n= 41, negative arm n= 10; ERα negative tumors n= 59, HSD17B1 positive arm n= 41, negative arm n= 18. P values were obtained from log-rank test.
Table 1. Characteristics by HSD17β1 expression in patients with ovarian tumors

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=111)</th>
<th>HSD17β1+ (n=82)</th>
<th>HSD17β1- (n=28)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>48.0</td>
<td>48.0</td>
<td>48.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borderline tumors</td>
<td>30/111 (27.0)</td>
<td>25/82 (30.5)</td>
<td>5/28 (17.9)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LGSC</td>
<td>10/111 (9.0)</td>
<td>8/82 (9.8)</td>
<td>2/28 (7.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HGSC</td>
<td>30/111 (27.0)</td>
<td>19/82 (23.2)</td>
<td>11/28 (39.3)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>25/111 (22.5)</td>
<td>18/82 (22.0)</td>
<td>7/28 (25.0)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Mucinous</td>
<td>9/111 (8.1)</td>
<td>6/82 (7.3)</td>
<td>3/28 (10.7)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Clear Cells</td>
<td>5/111 (4.5)</td>
<td>5/82 (6.1)</td>
<td>0/28 -</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Others</td>
<td>1/111 (0.9)</td>
<td>1/82 (1.2)</td>
<td>0/28 -</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>FIGO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>58/110 (52.7)</td>
<td>45/81 (55.6)</td>
<td>13/28 (46.4)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>II</td>
<td>4/110 (3.6)</td>
<td>2/81 (2.5)</td>
<td>2/28 (7.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>III</td>
<td>36/110 (32.7)</td>
<td>28/81 (34.6)</td>
<td>8/28 (28.6)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IV</td>
<td>11/110 (10.0)</td>
<td>6/81 (7.4)</td>
<td>5/28 (17.9)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Reproductive status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>56/107 (52.3)</td>
<td>39/79 (49.4)</td>
<td>17/27 (63.0)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Surgery debulking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimum</td>
<td>85/102 (83.3)</td>
<td>64/75 (85.3)</td>
<td>21/26 (80.0)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Protein expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatase (+)</td>
<td>72/110 (65.5)</td>
<td>54/82 (65.9)</td>
<td>18/28 (64.3)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ER α (+)</td>
<td>52/111 (46.8)</td>
<td>41/82 (50.0)</td>
<td>10/28 (35.7)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

HGSC high grade serous carcinoma, LGSC low grade serous carcinoma
Table 2. Frequency of positive reaction of HSD17β1, P450arom and ERα in histological subtypes of epithelial ovarian tumor

<table>
<thead>
<tr>
<th>Enzyme/Receptor</th>
<th>Borderline tumors</th>
<th>LGSC</th>
<th>HGSC</th>
<th>Endometrioid</th>
<th>Mucinous</th>
<th>Clear cells</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSD17β1</td>
<td>25/30 (83)</td>
<td>8/10 (80)</td>
<td>19/30 (63)</td>
<td>18/25 (72)</td>
<td>6/9 (67)</td>
<td>5/5 (100)</td>
<td>0.367</td>
</tr>
<tr>
<td>Aromatase</td>
<td>23/30 (77)</td>
<td>3/10 (30)</td>
<td>20/30 (67)</td>
<td>15/25 (60)</td>
<td>7/9 (78)</td>
<td>3/5 (60)</td>
<td>0.145</td>
</tr>
<tr>
<td>ERα</td>
<td>16/31 (52)</td>
<td>5/10 (50)</td>
<td>13/30 (43)</td>
<td>13/25 (52)</td>
<td>2/9 (22)</td>
<td>3/5 (60)</td>
<td>0.659</td>
</tr>
</tbody>
</table>

HGSC high grade serous carcinoma, LGSC low grade serous carcinoma
Percentage in parentheses
Table 3. Frequency of positive reaction of HSD17β1, P450arom and ERα according to clinical stages (FIGO)

<table>
<thead>
<tr>
<th>Enzyme/Receptor</th>
<th>EC I</th>
<th>EC II</th>
<th>EC III</th>
<th>EC IV</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSD17β1</td>
<td>44/57 (77)</td>
<td>2/4 (50)</td>
<td>28/36 (78)</td>
<td>6/11 (55)</td>
<td>0.268</td>
</tr>
<tr>
<td>Aromatase</td>
<td>35/57 (61)</td>
<td>3/4 (75)</td>
<td>24/36 (67)</td>
<td>8/11 (73)</td>
<td>0.903</td>
</tr>
<tr>
<td>ERα</td>
<td>26/57 (46)</td>
<td>4/4 (100)</td>
<td>17/37 (46)</td>
<td>5/11 (45)</td>
<td>0.208</td>
</tr>
</tbody>
</table>

Percentage in parentheses
Table 4. Cox proportional regression analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>1.04</td>
<td>1.01 - 1.07</td>
</tr>
<tr>
<td>Clinical stage (III &amp; IV)</td>
<td>14.4</td>
<td>4.3 - 48.1</td>
</tr>
<tr>
<td>HSD17β1 (+)</td>
<td>0.44</td>
<td>0.24 - 0.9</td>
</tr>
<tr>
<td>Aromatase (+)</td>
<td>1.78</td>
<td>0.7 - 4.4</td>
</tr>
<tr>
<td>ERα (+)</td>
<td>0.66</td>
<td>0.3 - 1.4</td>
</tr>
</tbody>
</table>
Figure 1.
Immunohistochemistry for HSD17B1, aromatase and ERα in a-c) High grade serous carcinoma; d-f) Endometrioid carcinoma; g-i) Mucinous carcinoma; j-l) Serous borderline tumor. Photomicrographs were obtained from similar regions of triple-positive samples. HSD17B1 and aromatase reactivity are detected in the cytoplasm of epithelial cells, ERα is visualized in a nuclear location. Bars represent 50 µm.
Figure 2.
Survival curves of patients with epithelial ovarian tumor after Kaplan Meier analysis according to HSD17B1, ERα, and aromatase positive and negative expression. A) Whole cohort (n= 111), HSD17B1 positive n= 83, negative n= 28; Aromatase positive n= 72, negative n= 38; ERα positive n=51, negative n=59. B) Serous epithelial ovarian tumors (n= 62), HSD17B1 positive n= 45, negative n= 16; Aromatase positive n= 39, negative n= 22; ERα positive n= 30, negative n= 31. C) Non-serous ovarian tumors (n= 49), HSD17B1 positive n= 37, negative n= 12; Aromatase positive n= 33, negative n= 16; ERα positive n= 21, negative n= 28. P values were obtained from log-rank test.
Figure 3.
Survival curves for HSD17B1 in the whole cohort of epithelial ovarian cancer stratified according to aromatase and ERα expression. A) Aromatase positive tumors n=72, HSD17B1 positive arm n=54, negative arm n=18; aromatase negative tumors, n= 38 HSD17B1 positive arm n= 28, negative arm n= 10; B) ERα positive tumors n= 51, HSD17B1 positive arm n= 41, negative arm n= 10; ERα negative tumors n= 59, HSD17B1 positive arm n= 41, negative arm n= 18. P values were obtained from log-rank test.