SPANDIDOS PUBLICATIONS

# Analysis of the expression of estrogen receptor, progesterone receptor and chicken ovalbumin upstream promoter-transcription factor I in ovarian epithelial cancers and normal ovaries

ROBÉRIO DE SOUSA DAMIÃO[1], CELINA TIZUKO FUJIYAMA OSHIMA[2], JOÃO NORBERTO STÁVALE[2] and WAGNER JOSÉ GONÇALVES[1]

[1]Discipline of Gynecologic Oncology, Department of Gynecology, and [2]Department of Pathology, Federal University of São Paulo, São Paulo, Brazil

Received March 3, 2007; Accepted April 10, 2007

**Abstract.** Sex hormones are involved in the carcinogenesis of some gynecologic cancers, and the status of their receptors represents an indicator of prognosis and of the therapeutic response in breast and endometrial cancers. In the ovary, this role is not clearly defined, with epithelial cancers being poorly responsive to hormone therapy. COUP-TFI (chicken ovalbumin upstream promoter-transcription factor I) is an orphan nuclear receptor, which is expressed in various tissues and regulates the estrogen receptor (ER) by competition for DNA binding. To investigate the role of these receptors in ovarian carcinogenesis and their implications for cancer prognosis, we evaluated the immunohistochemical expression of ER, progesterone receptor (PR) and COUP-TFI in benign and malignant ovarian epithelial neoplasms and in normal ovaries. A total of 113 ovarian specimens, including 40 diagnosed as malignant epithelial neoplasms (group A), 45 as benign epithelial tumors (group B), and 28 from normal ovaries (group C) were analyzed. Immunoexpression of ER was observed in 70% of patients of group A, 57.8% of group B and 57.1% of group C, with no significant difference between groups (p=0.426). Immunoexpression of PR was significantly lower in group A (12.5%) compared to group B (42.2%) and group C (32.1%) (p=0.010). Similarly, COUP-TFI was expressed in only 10% of group A patients, a rate significantly lower than that observed for group B (31.1%) and group C (39.3%) (p=0.014). No association was observed between the expression of these markers and increased survival or clinical prognostic variables. Multivariate analysis revealed a residual tumor <1 cm as the most significant clinical prognostic factor in group A (p=0.010, OR=4.14). These data support the importance of cytoreduction in the treatment of ovarian cancer, the role of steroid receptors in the mechanism of carcinogenesis, and the need for selection of subgroups that may respond to hormone therapy.

*Correspondence to*: Dr Wagner José Gonçalves, Rua Botucatu, 572 Cj. 42, Vila Clementino, São Paulo/SP, CEP: 04023-061, Brasil
E-mail: wj-goncalves@uol.com.br

*Key words:* estrogen receptor, progesterone receptor, chicken ovalbumin upstream promoter-transcription factor I, EAR3, ovarian cancer

## Introduction

Ovarian cancer is the fourth most frequent cause of cancer death in Western countries (1). It is characterized by typical aggressiveness and peritoneal invasion (2), with 90% of these tumors arising from epithelial cells of the ovarian surface (3). Most patients present advanced disease at the time of diagnosis because of poor symptomatology and the lack of screening methods (4).

Estrogen and progesterone and their receptors have been suggested to be involved in the carcinogenesis of various gynecologic cancers. Steroid receptor status has been implicated as an indicator of prognosis and therapeutic response in breast (5), endometrial and prostate cancers (6,7). However, the etiological factors involved in ovarian carcinogenesis have not been clearly established (8).

Recent epidemiological studies indicate estrogen as a possible promoter of ovarian cancer in postmenopausal women (8). Estrogen acts through two classical nuclear receptors, estrogen receptors α and ß (ERα and ERß), which are highly expressed in normal human ovaries and in benign, borderline and malignant ovarian tumors (9). Estrogen plays an important role in the proliferative stimulation of ovarian surface epithelial cells (10), promoting growth and inhibiting apoptosis (11). Prospective studies have demonstrated an increased risk for ovarian cancer in women receiving long-term hormone replacement therapy (12,13-15).

Ovarian cancer is considered to be non-hormone dependent due to its poor clinical response to hormone therapy. About 5-18% of cases are sensitive to treatment with tamoxifen, although 60% of patients are positive for ER expression (12,16). In contrast to breast cancer, in the ovary the prognostic

value of hormone receptor status and its role as a marker of antiestrogen treatment response are not clearly defined (17).

The progesterone receptor (PR) is regulated by estrogen in a small portion of neoplastic cells (18). In ovarian tumors, PR is preferentially expressed in the stromal fraction and positivity is observed in 30% of cases (19). However, its correlation as a prognostic marker has not been convincing (16).

Both ERα and ERß mediate the transcriptional activity of estrogen under the influence of regulatory molecules known as co-activators and co-repressors that activate or repress gene transcription (20).

COUP-TFs (chicken ovalbumin upstream promoter-transcription factors) are orphan nuclear receptors that are highly expressed during development of the nervous system (21) and in a wide variety of tissues such as liver, uterus, breast and ovary. These receptors regulate vital biological functions and organogenesis (22). The first member of this group of receptors, human COUP-TFI, was characterized as a transcription factor that binds to the COUP element which, in turn, regulates the transcription of the ovalbumin gene (23-25). Cloned as protein 3 related to v-ErbA, COUP-TFI is also known as EAR-3 (26). Its ability to specifically bind to the estrogen response element (ERE) and its half-sites suggests that COUP-TFI regulates the action of ER by direct DNA-binding competition and interaction with regulatory proteins (27). COUP-TFI might be implicated in ER inhibition by interacting with a DNA-binding region that overlaps with the ERE (28). The heterodimeric form of COUP-TFI bound to DNA with retinoic X receptor represents a universal pattern for many nuclear receptors (29) which modulate the hormonal response of a large number of genes (30,31). COUP-TFI has also been shown to serve as an accessory factor in the binding of some nuclear receptors to their ligands, suggesting that it may modulate these receptors both positively and negatively in a wide range of hormonal responses (32). The integration of multiple signaling pathways by the nuclear COUP-TFI receptor has repercussions on a large number of biological processes including cellular growth, differentiation and apoptosis (21,22,33,34).

The estrogen-ER complex activates transcription by binding to the ERE in the promoter of the target gene (35,36). Similarly, orphan nuclear receptors may modulate transcription by sharing target genes, co-regulatory proteins and DNA binding sites with the ER (36,37).

To investigate the role of these receptors in ovarian carcinogenesis and their relationship to cancer prognosis, we evaluated the immunohistochemical expression of ER, PR and COUP-TFI in benign and malignant ovarian neoplasms and in normal ovaries.

## Patients and methods

A prospective study was conducted on patients seen at the Discipline of Gynecologic Oncology, Federal University of São Paulo, between January 1994 and December 2004, who underwent exploratory laparotomy for adnexal tumors or prophylactic oophorectomy during surgery for a benign tumor of the uterine body. Patients with nonepithelial ovarian cancer or patients with a history of some type of malignant tumor were excluded. The clinical data were obtained during preoperative and postoperative visits. All surgeries were performed by the same team of researchers who collected 113 ovarian specimens over the study period, including 40 diagnosed as malignant epithelial neoplasms (group A), 45 as benign epithelial tumors (group B), and 28 from normal ovaries (group C). The study was approved by the Institutional Ethics Committee and all patients signed an informed consent form.

The following data were recorded upon anamnesis: age, race, age at menarche and menopause, postmenopausal duration, number of pregnancies, deliveries and abortions, and previous use of oral contraceptives. Weight, height and body mass index were recorded upon physical examination (38). Tumor-related data included histological type, degree of differentiation, size of the residual tumor after the first surgery, staging, and time of survival.

The histopathological diagnosis of the specimens obtained during surgery was made according to the criteria of the World Health Organization regarding degree of differentiation and to the criteria of the Gynecologic Oncology Group, and staging was performed according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) (39,40). The specimens were fixed in 10% formalin and embedded in paraffin. The slides were examined by two pathologists and selected based on the most representative areas.

Group A patients were staged and treated according to the FIGO criteria (39). In 12 (30%) patients the tumor was restricted to the ovaries (stages IA and IB) and the patients were submitted to hysterectomy, bilateral salpingo-oophorectomy, omentectomy and lymphadenectomy. The other 28 (70%) patients presented stages above stage IC and were submitted to cytoreductive surgery and adjuvant chemotherapy consisting of six cycles of cisplatin and paclitaxel. The extent of cytoreduction was classified as optimal when the residual tumor measured <1 cm or as suboptimal when the residual tumor was >1 cm (41,42).

*Anatomopathological analysis*. The surgical specimens were immediately placed in flasks containing 10% formalin, dehydrated in increasing ethanol concentrations, cleared in xylene, and embedded in paraffin. The blocks were cut into ~3-$\mu$m thick sections and the sections were mounted on slides. The slides were stained with hematoxylin and eosin and the histological type, degree of differentiation and the most representative areas were determined. For each case, triplicate 3-$\mu$m thick sections of the selected area were obtained and mounted on silanized slides for immunohistochemical analysis.

*Immunohistochemistry*. The sections (3-$\mu$m) were deparaffinized and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. Antigen retrieval was performed by microwave treatment in 10 mM citrate buffer, pH 6.0. The slides were then incubated at room temperature for 1 h with a monoclonal antibody against ER (1:50; Novocastra Laboratories, Newcastle upon Tyne, UK) and PR (1:30; Lab Vision, Fremont, CA, USA) and polyclonal antibody against COUP-TFI (1:100; Santa Cruz Biotechnology,

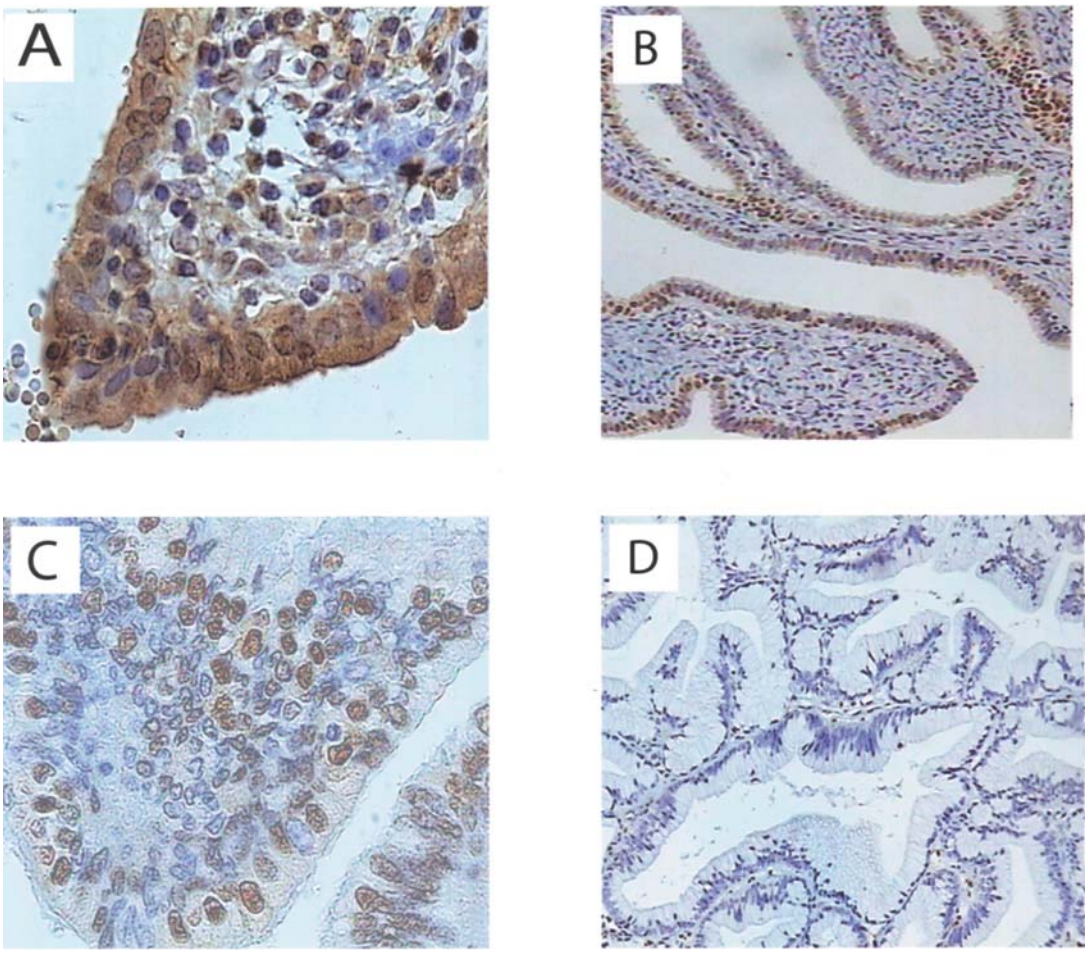


Figure 1. Positive immunostaining for the estrogen receptor (A), progesterone receptor (B), COUP-TFI (C), and negative control (D).

Santa Cruz, CA, USA). Next, the slides were incubated with the secondary biotinylated antibody for 20 min, followed by incubation with streptavidin-peroxidase solution for 20 min. The reaction was developed with 0.05% 3',3-diaminobenzidine tetrahydrochloride for 5 min, followed by washing in 0.05 M Tris buffer, pH 7.6, containing 0.024% hydrogen peroxide. The specimens were then counterstained with hematoxylin, dehydrated and mounted on slides. The biotinylated secondary antibodies and the streptavidin-peroxidase complex were diluted in phosphate-buffered saline, pH 7.4, containing 1% bovine serum albumin.

Breast carcinoma tissue was used as a positive control. The negative control consisted of omitting the primary antibody in phosphate-buffered saline.

Two observers independently evaluated the immunostaining intensity, with each of them counting at least 1000 tumor cells in about 10 random fields at high magnification. The results were expressed as percentage of tumor cells exhibiting a brown nuclear staining characteristic for the receptor and were scored as follows: negative (0), weak (<9%), moderate (10-50%), and strong (>50%). Samples with >10% of tumor cells showing the characteristic brown staining of the receptor were considered to be positive. The mean results of the two observers were considered in each case and immunostaining intensity was not considered (Fig. 1).

*Statistical analysis*. Qualitative variables are reported as absolute (n) and relative frequency (%), and quantitative variables are reported as mean, standard deviation, median, and range. The Kolmogorov-Smirnov test was applied to determine whether quantitative parameters presented a normal distribution. Quantitative variables were compared between groups by analysis of variance (ANOVA) when they presented a normal distribution; otherwise, the nonparametric Kruskal-Wallis test was used. The association between qualitative variables was evaluated by the Chi-square test or the Fisher's exact test. Agreement between expression levels was studied by the McNemar test. The Kaplan-Meier method was used to determine the effect of each factor on patient survival. Survival curves obtained for the categories of each factor were compared by the log-rank test. Cox regression analysis was used to determine the effect of the factors as a whole on patient survival. The odds ratio (OR) was calculated to evaluate the extent of the effect of significant factors on the occurrence of death. The level of significance was set at $p<0.05$ for all tests.

## Results

The mean age of the patients was 55.8 years (range, 20-87) in group A (malignant), 48.5 years (13-77) in group B (benign),

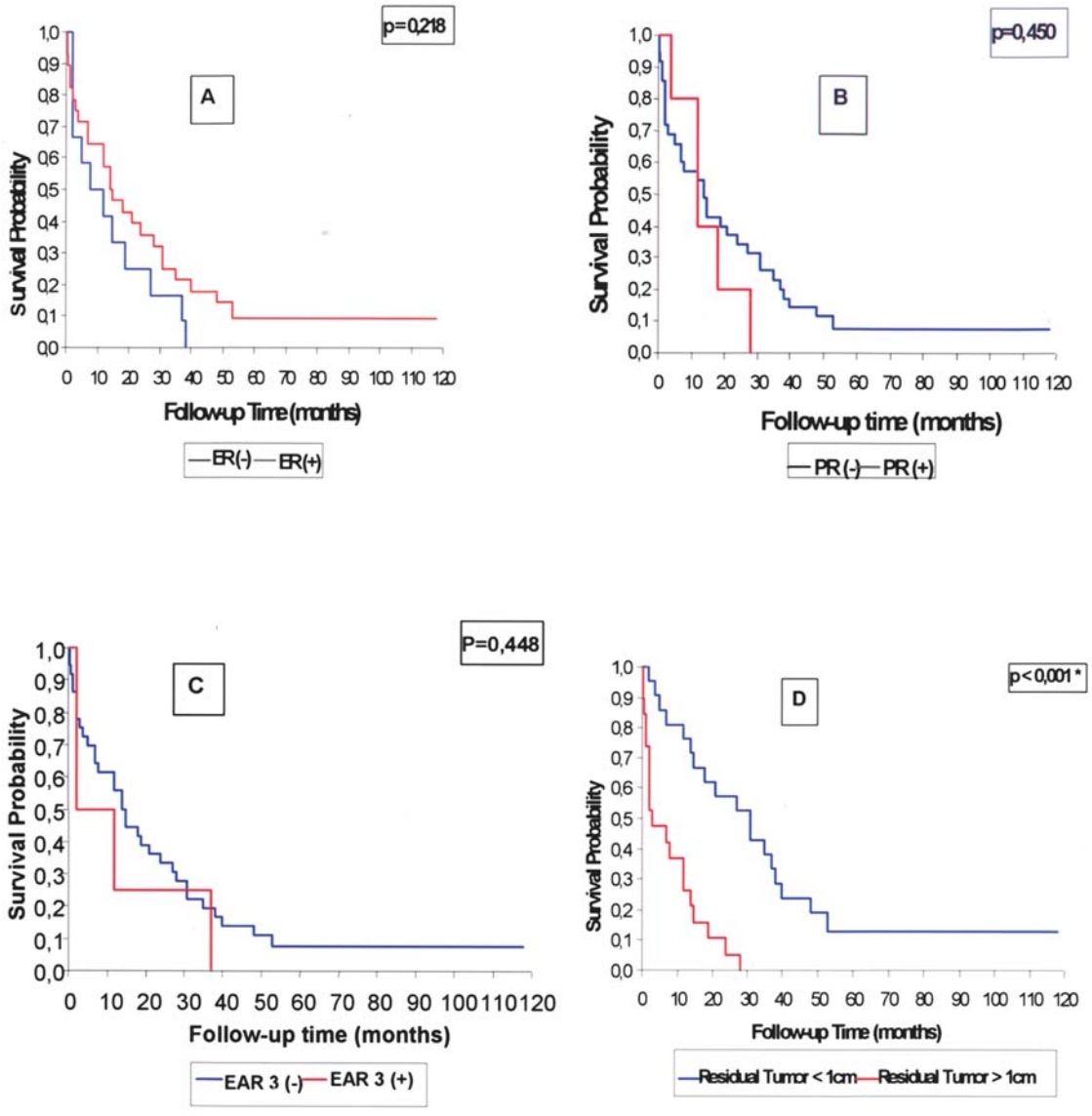


Figure 2. Association between estrogen receptor (A), progesterone receptor (B), COUP-TFI (EAR3) (C), residual tumor size (D) and survival of patients with ovarian epithelial carcinoma (Kaplan-Meier method).

and 53.1 years (25-80) in group C (normal), with no significant difference between groups (p=0.104). The groups were considered to be homogenous in terms of the distribution of the following variables: race (p=0.609), age at menarche (p=0.398), number of pregnancies (p=0.313), number of deliveries (p=0.418), number of abortions (p=0.154), previous use of oral contraceptives (p=0.847), age at menopause (p=0.728), menopausal status (p=0.692), and body mass index (p=0.752).

No significant association was observed between ER expression and patient group (p=0.426), with ER positivity being observed in 70% of group A, 57.8% of group B, and 57.1% of group C. There was a significant association between PR expression and patient group (p=0.010), with the proportion of PR-positive women being significantly lower in group A (12.5%) compared to groups B (42.2%) and C (32.1%) which did not differ from one another. A significant association was also observed between the expression of EAR3 and patient group (p=0.014), with the proportion of EAR3-positive women being significantly lower in group A (10%) compared to groups B (31.1%) and C (39.3%) which did not differ from one another.

Analysis of the relationship between ER and EAR3 expression showed an inverse expression in 80% of group A patients, 75.6% of group B and 67.9% of group C, with no significant difference between proportions (p=0.520). An inverse relationship between PR and EAR3 expression was observed in 17.5% of patients of group A, 42.2% of group B and 57.1% of group C, with a significant difference (p=0.003) between group A and groups B and C, which did not differ from one another. Analysis of the relationship between ER and PR expression showed an inverse expression in 62.5% of patients of group A, 37.8% of group B and 39.2% of group C. Comparison of these proportions demonstrated a significant difference (p=0.048) between group A and groups B and C, with the last two groups not differing from one another.

In group A, analysis of the association between receptor expression and classical prognostic variables showed no association between ER, PR or EAR3 expression and stage (p=0.477, p=0.633, p=1.000), residual tumor size (p=0.494,

Multivariate analysis (Cox regression) of survival in patients with ovarian epithelial carcinoma.

| Variable | Regression coefficient | SEM | p | OR | 95% CI |
|---|---|---|---|---|---|
| Estrogen receptor expression | 0.338 | 0.467 | 0.470 | 1.402 | (0.561, 3.504) |
| Progesterone receptor expression | 0.064 | 0.544 | 0.906 | 1.067 | (0.367, 3.100) |
| EAR3 expression | 0.011 | 0.697 | 0.988 | 1.011 | (0.258, 3.961) |
| Stage | 0.220 | 0.598 | 0.713 | 1.246 | (0.386, 4.020) |
| Residual tumor size | 1.423 | 0.552 | 0.010* | 4.148 | (1.405, 12.243) |
| Degree of differentiation | 0.063 | 0.449 | 0.889 | 1.065 | (0.442, 2.568) |

SEM, standard error of the mean; p, level of significance; OR, odds ratio; and 95% CI, 95% confidence interval.

p=1.000, p=0.331) or degree of differentiation (p=0.720, p=1.000, p=1.000), respectively. Also in group A, no association was observed between ER, PR or EAR3 expression and longer survival time (p=0.218, p=0.450, p=0.448, respectively) (Fig. 2). Comparison of the survival curves in group A according to tumor stage showed increased survival in patients with stages I/II compared to those with stages III/IV (p=0.004). Comparison of survival curves according to residual tumor size showed an increased survival of patients with a residual tumor <1 cm compared to those with a residual tumor >1 cm (p<0.001) (Fig. 2), whereas no significant differences between these curves were observed when analyzed according to the degree of differentiation (p=0.150).

Multivariate analysis by Cox regression comparing positive expression of ER, PR and EAR3, classical prognostic variables and survival time indicated the size of the primary postoperative residual tumor to be the most significant variable (p=0.010), with the chance of death being four times higher for patients with a residual tumor >1 cm (OR=4.148) (Table I).

## Discussion

Most ovarian epithelial carcinomas arise from the ovarian surface epithelium (12,43). There are numerous situations in which this epithelium presents a proliferative response to estrogen stimulation (10,11). In this respect, recent reports have suggested a role of estrogen in ovarian carcinogenesis (44) mainly based on the results of three large prospective studies which demonstrated an increased risk for ovarian cancer in women receiving long-term hormone replacement therapy (13-15). Some investigators believe that incessant ovulation may lead to chromosome instability and represents a potential risk for malignant transformation (45), with the epithelium being exposed to high concentrations of estrogen in the stroma and follicular fluid (46,47). The estrogen response depends, among other factors, on the expression of nuclear receptors and represents the central question in ovarian carcinogenesis whose underlying cellular mechanism has not been clearly established (9,48,49).

We observed no significant difference in ER expression between groups. Similar to other studies (16,50,51), we found high ER expression rates in normal ovaries (57.1%), benign epithelial tumors (57.8%) and malignant epithelial neoplasms (70%), and we understand this fact to be one of the multiple factors necessary for the action of estrogen in carcinogenesis. One may speculate that ER overexpression might imply a poorer prognosis of ovarian cancer; however, in agreement with other investigators (50,52) this was not observed in the present study, with no significant differences between the survival curves of ER-positive and ER-negative patients (p=0.218).

At the same time, epidemiological studies have shown that progesterone and its receptors protect against ovarian cancer since pregnant women and patients using contraceptives present a lower risk for this cancer (10,46). This fact can be attributed to the reduced exposure of the ovarian surface epithelium to estrogen as a consequence of ovulation suppression and of the effect of progesterone on cellular growth and apoptosis (46,53). The protective role of the PR was emphasized by the observation that patients showing a reduced expression of this receptor due to loss of heterozygosity at chromosome 11q23.3-24.3 present a higher risk of ovarian cancer and a poorer prognosis (54). Progesterone inhibits cell proliferation, stimulates the expression of p53 and induces apoptosis in breast cancer and ovarian cells, suggesting a protective role in carcinogenesis (55,56). In the present study, PR expression was significantly lower in the malignant group (12.5%) compared to the benign and normal groups, a fact that might be related to the loss of the protective capacity of this receptor during carcinogenesis. The cause of the loss of PR expression in ovarian epithelial carcinoma is unknown (57), but might be related to a reduced response of ovarian cancer cells to estrogen (58) or, secondarily, to loss of heterozygosis (59,60); however, somatic mutations in the PR sequence are rare (10).

The antineoplastic effect of the PR is represented by a better survival of PR-positive patients with ovarian cancer (70). This fact was not confirmed in the present study since no significant differences were observed in the survival curves of patients with positive and negative receptor expression

(p=0.450). Some studies demonstrated an association between PR expression and a longer disease-free interval (19,51,61-63), whereas others did not (64-66). These conflicting data seem to be due to various factors such as the method used for receptor detection, criteria for histological quantification, selection of cases, and sample size.

Both ERα and ERß mediate the transcriptional activity of estrogen by influencing regulatory molecules such as co-activators and co-repressors that activate or repress the transcriptional activity of genes responsive to estrogen (20). The mechanism of resistance to hormone therapy remains unknown but it is believed that these co-factors may influence the hormone response by regulating the interaction of the receptor-steroid hormone complex and the transcriptional regulatory element in DNA, inhibiting or activating transcriptional activity (51). One possible explanation for the loss of ER responsiveness is inactivation due to mutations. However, analysis of the ER in ovarian carcinomas suggests the occurrence of variants but no inactivation has been detected (67).

The observation of COUP-TFI expression in 10% of malignant cases, a rate significantly lower than that observed for the other groups (p=0.014), suggests that the loss of expression of this factor might be related to carcinogenesis, especially when considering its inhibitory activity on the ER. COUP-TFI has been highly conserved during evolution and is involved in different biological functions such as the repression of gene expression (30) through interaction with co-repressors (68). Some investigators demonstrated that the direct interaction between ER and COUP-TFI is influenced by the ER ligand and becomes more intense when ER is liganded with antiestrogens such as tamoxifen (69). COUP-TFI and ER form a complex that interacts with co-repressors, recruiting them and stabilizing the binding, with the consequent transcriptional repression of estrogen-responsive genes (69). The expression of these genes is not only modulated by the ER-ERE interaction but also by the interaction with transcription factors and orphan receptors including COUP-TFI (27), whose ligands are unknown (69).

The different possibilities of COUP-TFI binding to DNA and its ability to compete with other nuclear receptors for the same hormone response element confer on this marker a negative regulatory function for a large number of genes (22,29,30). COUP-TFI has been implicated in ER inhibition based on the binding to the lactoferrin and ocytocin promoters which overlap the ERE (28). The ability of COUP-TFI to bind to ERE half-sites, inhibiting estradiol-induced gene expression, suggests that this factor regulates the action of the ER by competing for direct DNA binding and by interaction with regulatory proteins (27). The integration of multiple signaling pathways by COUP-TFI has repercussions on a large number of biological processes related to cellular growth, differentiation, embryogenesis and apoptosis (21,22,33,34). Thus, we suggest that one of the mechanisms explaining the variety in the response to estrogen is the transcriptional relationship between ER and nuclear receptors such as COUP-TFI as demonstrated in part by the co-existence of these receptors on epithelial cells of the uterus, ovary, kidney, prostate and liver (21,22,70). Furthermore, no differences in survival curves related to COUP-TFI expression were observed in the present study.

The significantly higher proportion of an inverse expression of ER and PR in the malignant group (p=0.048) supports the hypothesis of an antagonistic role of these receptors in the process of carcinogenesis, a fact not observed for ER and EAR3 (p=0.520). In contrast, the significantly lower proportion of an inverse expression of PR and EAR3 in the malignant group (p=0.003) supports the hypothesis of a protective role of these receptors in the process of carcinogenesis.

No association between the expression of these receptors, classical prognostic variables and survival time was observed in the malignant group. However, multivariate analysis indicated residual tumor size as the most significant clinical predictor of survival (p=0.010), with the chance of death being four times higher for patients with a residual tumor >1 cm (OR=4.148) (Table I).

These data support the importance of cytoreduction in the treatment of ovarian cancer, the role of steroid receptors in the mechanism of carcinogenesis, and the need for selection of subgroups which might respond to hormone therapy. The understanding of the molecular mechanisms underlying the resistance of ovarian cancer to antiestrogen therapy will certainly be of help in the establishment of a strategy to overcome this dilemma. New selective modulators that specifically block the activity of ERα and its regulators should be explored in an attempt to guarantee the success of antiestrogen therapy in ovarian cancer.

## Acknowledgements

We thank all members of the Disciplines of Gynecologic Oncology and Pathological Anatomy, UNIFESP, for their direct and indirect collaboration with this study.

## References

1. Greenlee RT, Murray T, Bolden S and Wingo PA: Cancer statistics, 2000. CA Cancer J Clin 50: 7-33, 2000.
2. Katso RM, Manek S, O'Byrne K, Playford MP, Lê Meuth V and Ganesan TS: Molecular approaches to diagnosis and management of ovarian cancer. Cancer Metastasis Rev 16: 81-107, 1997.
3. Auersperg N, Wong AS, Choi KC, Kang SK and Leung PC: Ovarian surface epithelium: biology, endocrinology, and pathology. Endocr Rev 22: 255-288, 2001.
4. Davies BR, Auersperg N, Worsley SD and Ponder BA: Transfection of rat ovarian surface epithelium with erb-B2/neu induces transformed phenotypes *in vitro* and the tumorigenic phenotype *in vivo*. Am J Pathol 152: 297-306, 1998.
5. Mason BH, Holdaway IM, Mullins PR, Yee LH and Kay RG: Progesterone and estrogen receptors as prognostic variables in breast cancer. Cancer Res 43: 2985-2990, 1983.
6. Iwail K, Fukuda K, Hachisuga T, Mori M, Uchiyama M, Iwasaka T, *et al*: Prognostic significance of progesterone receptor immunohistochemistry for lymph node metastasis in endometrial carcinoma. Gynecol Oncol 72: 351-359, 1999.
7. Nativ O, Umehara T, Colvard DS, Therneau TM, Farrow GM, Spelsberg TC, *et al*: Relationship between DNA ploidy and functional estrogen receptors in operable prostate cancer. Eur Urol 32: 96-99, 1997.
8. Cunat S, Hoffmann P and Pujol P: Estrogens and epithelial ovarian cancer. Gynecol Oncol 94: 25-32, 2004.
9. Muramatsu M and Inoue S: Estrogen receptors: how do they control reproductive and nonreproductive functions? Biochem Biophys Res Commun 270: 1-10, 2000.

1: Estrogen, progesterone and epithelial ovarian cancer. J Biol Endocrinol 1: 73, 2003.
11. Syed V, Ulinski G, Mok SC, Yiu GK and Ho SM: Expression of gonadotropin receptor and growth responses to key reproductive hormones in normal and malignant human ovarian surface epithelial cells. Cancer Res 61: 6768-6776, 2001.
12. Hatch KD, Beecham JB, Blessing JA and Creasman WT: Responsiveness of patients with advanced ovarian carcinoma to tamoxifen. A Gynecologic Oncology Group study of second-line therapy in 105 patients. Cancer 68: 269-271, 1991.
13. Anderson GL, Judd HL, Kaunitz AM, *et al*: Effects of estrogen plus progestin on gynecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial. JAMA 290: 1739-1748, 2003.
14. Lacey JV Jr, Mink PJ, Lubin JH, *et al*: Menopausal hormone replacement therapy and risk of ovarian cancer. JAMA 288: 334-341, 2002.
15. Rodriguez C, Patel AV, Calle EE, Jacob EJ and Thun MJ: Estrogen replacement therapy and ovarian cancer mortality in a large prospective study of US women. JAMA 285: 1460-1465, 2001.
16. Rao BR and Slotman BJ: Endocrine factors in common epithelial ovarian cancer. Endocr Rev 12: 14-26, 1991.
17. Perez-Gracia JL and Carrasco EM: Tamoxifen therapy for ovarian cancer in the adjuvant and advanced settings: systematic review of the literature and implications for future research. Gynecol Oncol 84: 201-209, 2002.
18. Langdon SP, Hirst GL, Miller EP, Hawkins RA, Tesdale AL, Smyth JF, *et al*: The regulation of growth and protein expression by estrogen *in vitro*: a study of 8 human ovarian carcinoma cell lines. J Steroid Biochem Mol Biol 50: 131-135, 1994.
19. Kommoss F, Pfisterer J, Thome M, Schafer W, Sauerbrei W and Pfleiderer A: Steroid receptors in ovarian carcinoma: immunohistochemical determination may lead to new aspects. Gynecol Oncol 47: 317-322, 1992.
20. Hussein-Fikret S and Fuller PJ: Expression of nuclear receptor coregulators in ovarian stromal and epithelial tumours. Mol Cell Endocrinol 229: 149-160, 2005.
21. Qiu Y, Pereira FA, de Mayo FJ, Lydon JP, Tsai SY and Tsai MJ: Null mutation of mCOUP-TFI results in defects in morphogenesis of the glossopharyngeal ganaxonal projection and arborization. Genes Dev 11: 1925-1937, 1997.
22. Pereira FA, Qiu Y, Tsai MJ and Tsai SY: Chicken ovalbumin upstream promoter transcription factor (COUP-TF): expression during mouse embryogenesis. J Steroid Biochem Mol Biol 53: 503-508, 1995.
23. Sagami I, Tsai SY, Wang H, Tsai ML and O'Malley BW: Identification of two factors required for transcription of the ovalbumin gene. Mol Cell Biol 6: 4259-4267, 1986.
24. Wang LH, Tsai SY, Sagami L, Tsai ML and O'Malley BW: Purification and characterization of chicken ovalbumin upstream promoter transcription factor from HeLa cells. J Biol Chem 262: 16080-16086, 1987.
25. Wang LH, Tsai SY, Cook RG, Beattie WG, Tsai ML and O'Malley BW: CC transcription factor is a member of the steroid receptor superfamily. Nature 340: 163-166, 1989.
26. Miyajima N, Kadowaki Y, Fukushige S, Shimizu S, Semba K, Yamanashi Y, *et al*: Identification of two novel members of erbA superfamily by molecular cloning: the gene products of the two are highly related to each other. Nucleic Acids Res 16: 11057-11074, 1988.
27. Klinge CM, Silver BF, Driscoll MD, Sathya G, Bambara RA and Hilf R: Chicken ovalbumin upstream promoter-transcription factor interacts with estrogen receptor, binds to estrogen response elements and half-sites, and inhibits estrogen-induced gene expression. J Biol Chem 272: 31465-31474, 1997.
28. Liu Y, Yang N and Teng CT: COUP-TF acts as a competitive repressor for estrogen receptor-mediated activation of the mouse lactoferrin gene. Mol Cell Biol 13: 1836-1846, 1993.
29. Cooney AJ, Tsai SY, O'Malley BW and Tsai MJ: Chicken ovalbumin upstream promoter transcription factor (COUP-TFI) dimers bind to different GGTCA response elements, allowing COUP-TF to repress hormonal induction of the vitamin D3, thyroid hormone, and retinoic acid receptors. Mol Cell Biol 12: 4153-4163, 1992.
30. Tsai SY and Tsai MJ: Chick ovalbumin upstream promoter-transcription factors (COUP-TFs): coming of age. Endocr Rev 18: 229-240, 1997.
31. Pereira FA, Tsai MJ and Tsai SY: COUP-TF orphan nuclear receptors in development and differentiation. Cell Mol Life Sci 57: 1388-1398, 2000.
32. Gay F, Barath P, Desbois-Le Peron C, Metivier R, Le Guevel R, Birse D and Salbert G: Multiple phosphorylation events control chicken ovalbumin upstream promoter transcription factor I orphan nuclear receptor activity. Mol Endocrinol 16: 1332-1351, 2002.
33. Lin B, Chen GQ, Xiao D, Kolluri SK, Cao X, Su H and Zhang XK: Orphan receptor COUP-TF is required for induction of retinoic acid receptor ß growth inhibition and apoptosis by retinoic acid in cancer cells. Mol Cell Biol 20: 957-970, 2000.
34. Metivier R, Gay FA, Hubner MR, Flouriot G, Salbert G, Gannon F, Kah O and Pakdel F: Formation of an hERalpha-COUP-TFI complex enhances hER alpha AF-1 through Ser 118 phosphorylation by MAPK. EMBO J 21: 3443-3453, 2002.
35. Clinton GM and Hua W: Estrogen action in human ovarian cancer. Crit Rev Oncol Hematol 25: 1-9, 1997.
36. Giguere V: Orphan nuclear receptors: from gene to function. Endocr Rev 20: 689-725, 1999.
37. Sun P, Sehouli J, Denkert C, Mustea A, Konsgen D, Koch I, Wei L and Lichtenegger W: Expression of estrogen receptor-related receptors, a subfamily of orphan nuclear receptors, as new tumor biomarker in ovarian cancer cells. J Mol Med 83: 457-467, 2005.
38. Keys A, Fidanza F, Karvonen MJ, Kimura N and Taylor HL: Indices of relative weight and obesity. J Chronic Dis 25: 329-343, 1972.
39. Scully RE: World Health Organization classification and nomenclature of ovarian cancer. Natl Cancer Inst Monogr 42: 5-7, 1975.
40. Shepherd JH: Revised FIGO staging for gynaecological cancer. Br J Obstet Gynaecol 96: 889-892, 1989.
41. Hoskins WJ: Epithelial ovarian carcinoma: principles of primary surgery. Gynecol Oncol 55: S91-S96, 1994.
42. Hoskins WJ, McGuire WP, Brady MF, Homesley HD, Creasman WT, Berman M, *et al*: The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with sub-optimal residual epithelial ovarian carcinoma. Am J Obstet Gynecol 170: 974-979, 1994.
43. Scully RE: Pathology of ovarian cancer precursors. J Cell Biochem 23: 208-218, 1995.
44. Riman T, Persson I and Nilsson S: Hormonal aspects of epithelial ovarian cancer: review of epidemiological evidence. Clin Endocr 49: 695-707, 1998.
45. Fathalla MF: Incessant ovulation - a factor in ovarian neoplasia? Lancet 2: 163, 1971.
46. Risch HA: Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. J Natl Cancer Inst 90: 1774-1786, 1998.
47. Lindgren PR, Backstrom T, Cajander S, *et al*: The pattern of estradiol and progesterone differs in serum and tissue of benign and malignant ovarian tumors. Int J Oncol 21: 583-589, 2002.
48. Bardin A, Hoffmann P, Boulle N, Katsaros D, Vignon F, Pujol P and Lazennec G: Involvement of estrogen receptor beta in ovarian carcinogenesis. Cancer Res 64: 5861-5869, 2004.
49. Li AJ, Baldwin RL and Karlan BY: Estrogen and progesterone receptor subtype expression in normal and malignant ovarian epithelial cell cultures. Am J Obstet Gynecol 89: 22-27, 2003.
50. Lee P, Rosen DG, Zhu C, Silva EG and Liu J: Expression of progesterone receptor is a favorable prognostic marker in ovarian cancer. Gynecol Oncol 96: 671-677, 2005.
51. Havrilesky LJ, McMahon CP, Lobenhofer EK, Whitaker R, Marks JR and Berchuck A: Relationship between expression of coactivators and corepressors of hormone receptors and resistance of ovarian cancers to growth regulation by steroid hormones. J Soc Gynecol Investig 8: 104-113, 2001.
52. Munstedt K, Steen J, Knauf AG, Buch T, von Georgi R and Franke FE: Steroid hormone receptors and long term survival in invasive ovarian cancer. Cancer 89: 1783-1791, 2000.
53. Syed V and Ho SM: Progesterone-induced apoptosis in immortalized normal and malignant human ovarian surface epithelial cells involves enhanced expression of fasL. Oncogene 22: 6883-6890, 2003.
54. Gabra H, Langdon SP, Watson JE, Hawkins RA, Cohen BB, Taylor L, *et al*: Loss of heterozygosity at 11q22 correlates with low progesterone receptor content in epithelial ovarian cancer. Clin Cancer Res 1: 945-953, 1995.
55. Formby B and Wiley TS: Progesterone inhibits growth and induces apoptosis in breast cancer cells: inverse effects on Bcl-2 and p53. Ann Clin Lab Sci 28: 360-369, 1998.

56. Bu SZ, Yin DL, Ren XH, Jiang LZ, Wu ZJ, Gao QR, *et al*: Progesterone induces apoptosis and up-regulation of p53 expression in human ovarian carcinoma cell lines. Cancer 79: 1944-1950, 1997.
57. Lindgren PR, Cajander S, Backstrom T, Gustafsson JA, Makela S and Olofsson JI: Estrogen and progesterone receptors in ovarian epithelial tumors. Mol Cell Endocrinol 221: 97-104, 2004.
58. Fujimura M, Hidaka T, Kataoka K, Yamakawa Y, Akada S, Teranishi A and Saito S: Absence of estrogen receptor-α expression in human ovarian clear cell adenocarcinoma compared with ovarian serous, endometrioid, and mucinous adenocarcinoma. Am J Surg Pathol 25: 667-672, 2001.
59. Gabra H, Taylor L, Cohen BB, Lessels A, Eccles DM, Leonard RC, Smyth JF and Steel CM: Chromosome 11 allele imbalance and clinicopathological correlates in ovarian tumours. Br J Cancer 72: 367-375, 1995.
60. Gabra H, Watson JE, Taylor KJ, Mackay J, Leonard RC, Steel CM, Porteous DJ and Smyth JF: Definition and refinement of a region of loss of heterozygosity at 11q23.3-q24.3 in epithelial ovarian cancer associated with poor prognosis. Cancer Res 56: 950-954, 1996.
61. Hempling RE, Piver MS, Eltabbakh GH and Recio FO: Progesterone receptor status is a significant prognostic variable of progression-free survival in advanced epithelial ovarian cancer. Am J Clin Oncol 21: 447-451, 1998.
62. Slotman BJ, Nauta JJ and Rao BR: Survival of patients with ovarian cancer. Apart from stage and grade, tumor progesterone receptor content is a prognostic indicator. Cancer 66: 740-744, 1990.
63. Akahira J, Inoue T, Suzuki T, Ito K, Konno R, Sato S, Moriya T, Okamura K, Yajima A and Sasano H: Progesterone receptor isoforms A and B in human epithelial ovarian carcinoma: immunohistochemical and RT-PCR studies. Br J Cancer 83: 1488-1494, 2000.
64. Fischer-Colbrie J, Witt A, Heinzl H, Speiser P, Czerwenka K, Sevelda P and Zeillinger R: EGFR and steroid receptors in ovarian carcinoma: comparison with prognostic parameters and outcome of patients. Anticancer Res 17: 613-619, 1997.
65. Goff BA, Ries JA, Els LP, Coltrera MD and Gown AM: Immunophenotype of ovarian cancer as predictor of clinical outcome: evaluation at primary surgery and second-look procedure. Gynecol Oncol 70: 378-385, 1998.
66. Lancaster JM, Wenham RM, Halabi S, Calingaert B, Marks JR, Moorman PG, Bentley RC, Berchuck A and Schildkraut JM: No relationship between ovarian cancer risk and progesterone receptor gene polymorphism in a population-based, case-control study in North Carolina. Cancer Epidemiol Biomarkers Prev 12: 226-227, 2003.
67. Tonetti DA and Jordan VC: The role of estrogen receptor mutations in tamoxifen-stimulated breast cancer. J Steroid Biochem Mol Biol 62: 119-128, 1997.
68. Shibata H, Nawaz Z, Tsai SY, O'Malley BW and Tsai MJ: Gene silencing by chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI) is mediated by transcriptional corepressors, nuclear receptor-corepressor (N-CoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT). Mol Endocrinol 11: 714-724, 1997.
69. Klinge CM: Role of estrogen receptor ligand and estrogen response element sequence on interaction with chicken ovalbumin upstream promoter transcription factor (COUP-TF). J Steroid Biochem Mol Biol 71: 1-19, 1999.
70. Shigeta H, Newbold RR, McLachlan JA and Teng C: Estrogenic effect on the expression of estrogen receptor, COUP-TF and lactoferrin mRNA in developing mouse tissues. Mol Reprod Dev 45: 21-30, 1996.