Progesterone receptor expression correlates with estrogen receptor α but not β and pS2 status in ovarian carcinomas: relation to apoptosis, cell proliferation, p27Kip1 expression, and survival

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Objective: Possible associations among the expression of the estrogen receptors (ER) α and β, progesterone receptor (PR), and pS2, as well as cell kinetics, in ovarian tumors were examined. Methods: A total of 120 carcinomas, along with 29 low malignant potential (LMP) tumors and 70 cystadenomas, were immunohistochemically investigated for ER, PR, and pS2 expressions. The results were subsequently compared to apoptosis, cell proliferation, and survival. The mRNA analyses were also examined. Results: ERα, ERβ, and PR expressions at both protein scores and mRNA levels were not altered among benign, LMP, or malignant lesions, while pS2 expression was detected in mucinous tumors only, because pS2 expression was inversely associated with ERα and PR. There were statistically significant correlations between the ERα and PR scores in all the categories, with the exception of some carcinomas; however, the ERβ scores were independent. There were no statistically significant correlations between hormone-related molecules and cell kinetic markers in any of the tumor categories. Relatively high PR scores in women with ovarian carcinomas, however, were significantly associated with favorable outcomes. Conclusions: These results suggest that ERα rather than ERβ may play important roles in the regulation of PR but not pS2 expressions in ovarian tumors. Moreover, independent of changes in tumor tissue kinetics, relatively high PR expression indicated good prognoses in women with ovarian cancer.

Key words: ERα, ERβ, progesterone receptor, pS2, ovarian carcinoma

Introduction

The question of whether or not ovarian carcinoma growth is hormone-dependent remains unanswered. While the growth of estrogen receptor (ER)-positive ovarian cancer cell lines sensitive to 17β-estradiol may be inhibited by antiestrogens, only a small proportion of lesions clinically respond to antiestrogen treatment, even though ER is expressed in approximately half of the cases, which corresponds with the findings for progesterone therapy. Moreover, although several studies have noted a link between hormone receptor status and the survival of patients with ovarian carcinomas, the published data are conflicting.

Recently, a new ER subtype, ERβ, has been identified in rat and mouse prostate and ovary, and in human thymus, spleen, ovary, and testis. Highly homologous to the classic form (ERα), in particular in its DNA and ligand binding domains, and a similar affinity to 17β-estradiol have been reported. Although previous studies have demonstrated alterations in ratios of mRNA expression for both ER subtypes during ovarian tumorigenesis, little is known about associations among ERα, ERβ, and progesterone receptor (PR) status.

The pS2 gene encodes a member of the trefoil factor family (TFF) group of small secretory peptides with 1-6 peptides of the highly conserved 6-cysteine-rich region, the expression of which is regulated by estrogen at the transcription levels. Although a significant link between pS2 and ER expression has been documented for breast and endometrial carcinomas, to our knowledge, there have been few investigations of pS2 expression in ovarian neoplasias.

In this study, to clarify possible associations among
expression of ER\(_{\alpha}\), ER\(_{\beta}\), PR, and pS2 in ovarian tumors, immunohistochemical and a combination of PCR and Southern blot hybridization (SBH) assays were applied to a series of benign, premalignant, and malignant lesions. The results were also compared with apoptosis, cell proliferation, p27\(^{kip1}\) expression, and survival.

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c\ls p\005ials and Methods

C\ls es
A total of 120 cases of ovarian carcinomas, surgically resected at Kitasato University Hospital from 1992 to 1999, were investigated, along with 29 low malignant potential (LMP) tumors and 70 cystadenomas. The ages of the patients ranged from 26 to 82 years with a mean of 55.3.

All tissues were obtained by oophorectomy, with or without hysterectomy, routinely fixed in 10% formalin, and processed for embedding in paraffin wax. Histological diagnoses were performed according to the criteria of the World Health Organization (WHO) and the International Federation of Gynecology and Obstetrics (FIGO). The tumors investigated comprised 52 serous, 18 mucinous, 16 endometrioid, and 34 clear cell carcinomas, as well as 7 serous and 22 mucinous LMP tumors, and 18 serous and 52 mucinous cystadenomas. Based on degree of histological differentiation, carcinomas were subclassified into 59 grade (G) 1, 36 G2, and 25 G3. Of carcinoma cases available for investigation of clinicopathological factors, 62 were categorized in stage I/II and 57 in stage III/IV, while 24 were positive and 50 negative for lymph node metastasis. Of carcinoma cases, 109 could be analyzed for their outcome after surgery, with a mean follow-up time of 2.6 years (range, 0.1-7.2 years). Some cases had received platinum-based chemotherapy after primary surgery.

Immunohistochemistry

Immunohistochemistry (IHC) was performed using a combination of microwave-oven heating and the standard labeled Streptavidin-biotin (LSAB kit; Dako, Cophenhagen, Denmark) methods. Biotinylated horse anti-goat IgG was also applied as the secondary antibody for detection of ER\(_{\beta}\) protein. After boiling in 10 mM Citrate buffer (pH 6.0) for two 10-minute cycles, slides were incubated with optimum dilution of primary antibodies. The antibodies employed were anti-ER\(_{\alpha}\) mouse monoclonal (Novocastra Lab, Newcastle, UK), anti-ER\(_{\beta}\) goat polyclonal (SantaCruze Biotech, Santa Cruz, USA), anti-PR mouse monoclonal (Novocastra Lab), anti-pS2 mouse monoclonal (Dako), anti-human Ki-67 antigen rabbit polyclonal (Dako), and anti-p27\(^{kip1}\) mouse monoclonal (Transduction Lab, Lexington, USA) antibodies.

Evaluation of the IHC results was carried out on the basis of both distribution of immunopositive epithelial cells and immunointensity, as described previously.\(^{18,19}\) Immunoreactivity scores were generated by multiplication of the values for the two parameters.

Apoptotic and mitotic indices (AI and MI), and Ki-67 and p27\(^{kip1}\) labeling indices (LIs)

Detection of apoptotic cells in hemotoxylin and eosin stained sections was performed, at high-power magnification, in accordance with the criteria of Kerr et al.\(^{20}\) AI values were calculated after examining at least 1,000 nuclei in 5 to 8 randomly selected fields for each case. Areas of severe inflammatory cell infiltration and necrosis were excluded, since some doubtful cells were observed in such lesions. MI and LIs of Ki-67 and p27\(^{kip1}\) were also examined in a similar manner.

RT-PCR and SBH assays

Total cellular RNAs were extracted and cDNA were synthesized in the presence of random primers. The PCR procedure was performed with 25 to 30 cycles. For detection of mRNA for both ER\(_{\alpha}\) and \(\beta\) genes, competitive RT-PCR assays were conducted according to the methods described by Pujol et al.\(^{11}\) using common forward primer (5’-AAGAGCTGCCAGGCGCTGCC-3’) for both genes and different reverse primers for ER\(_{\alpha}\) (5’-TTGGCAGCTCTCATGTCTCC-3’) and for ER\(_{\beta}\) (5’-GCGCACTGGGGCGGGCTGATCA-3’). Specific primers for PR (5’-GCTCCGAGCTCGGCTGAT-3’ and 5’-ACAGCTAGCTGCTTCCATCCC-3’) and for pS2 (5’-ACCATGGAGAACAGGTTGAT-3’ and 5’-AAATTCGACAAGGGGTTCTCG-3’) were also applied. For semi-quantitation of target genes, co-amplification with the \(\beta\)-actin gene was carried out. As negative control, water was used instead of template cDNA for each examination.

A 10\(\mu\)l aliquots of each PCR reaction mixture was electrophoresed and transferred to a Hybond-N+ nylon membrane (Amersham, Tokyo). Filters were hybridized overnight with digoxigenin-labeled specific probes for both target and \(\beta\)-actin genes. The sequences of the oligonucleotide probes were probe ER\(_{\alpha}\) (5’-
Figure 1. Immunoreactivity of hormone receptors and pS2 in ovarian carcinomas.
A. Estrogen receptor (ER)α in a serous carcinoma. B. ERβ in a mucinous carcinoma. C. Progesterone receptor (PR) in an endometrioid carcinoma. D. pS2 in a mucinous carcinoma. Note the negative reactions for these antibodies in stromal components, in contrast to the immunopositive epithelial cells. Original magnification, ×200.

Figure 2. Relation between immunoreactivity scores for hormone receptors and pS2 and histological tumor phenotypes. IHC, immunohistochemistry; LMP, low malignant potential tumor; S, serous type; M, mucinous type; E, endometrioid type; C, clear cell type; ER, estrogen receptor; PR, progesterone receptor. The data are mean ± SD values.
CTCGCAATGCTACGAAATGGGAATGATG-3'), probe ER β (5' - TGAGCCCCGACGCTAGTGCTACCCCTCC-3'), probe PR (5' - CCGAGGCTACTCCGCCCTATCTCAACTAC-3'), and probe pS2 (5' - GAAAGACAGAATTGTGGTTTTCCTGGTGTC-3'). Hybridization signals were detected using a DIG Luminescent Detection Kit (Roche Diagnostics, Mannheim, Germany). The conditions used for hybridization, washing, and detection were according to the manufacturers' recommendations. The sequence of primers and the oligonucleotide probe for the β-actin gene have been previously reported.21

Analysis of hybridization signals was achieved with NIH Image version 1.58 software. The relative amounts were calculated by normalization to the hybridization signals for β-actin in each case.

Statistics
Comparative data were analyzed using the Mann-Whitney U test and the Pearson’s correlation coefficient.

Survival was measured from time of the primary operation and survival curves were generated by the Kaplan-Meier method. The log-rank test and Cox proportional hazards modeling were performed to compare survival rates between classified subgroups. Values of P < 0.05 indicated statistical significance.

Results
Immunohistochemical findings
Immunoreactivity for ER α, ER β, and PR was evident as a distinct nuclear staining, with markedly heterogeneous distribution and variability in immunointensity in ovarian epithelial tumor lesions. Immunoreactions in some stromal components were also noted in benign and premalignant lesions but were rare in carcinomas. The antibody against pS2 protein mainly reacted with luminal surfaces, with or without cytoplasmic immunostaining, in mucinous ovarian tumors, while stromal components completely lacked any reaction (Figure 1).

Overall average values for ER α, ER β, and PR did not show any differences among benign, LMP, and malignant ovarian tumors, in contrast to pS2 values which were significantly lower in carcinomas (P < 0.0001, respectively), in line with decrease in the proportion with a mucinous phenotype.

In ovarian carcinomas, significantly high PR and pS2 values were noted in endometrioid and mucinous phenotypes compared with the other phenotypes, while there were no differences in average ER α or ER β values among histological categories. In cystadenomas and LMP tumors, the ER α and PR values were significantly higher in the serous phenotypes than those in the mucinous phenotypes, in contrast to the pS2 values, which were significantly higher in the mucinous phenotypes. No difference in ER β scores between histological phenotypes was noted (Figure 2).

As shown in Table 1, average ER α values positively correlated with PR scores in cystadenomas, LMP tumors, and endometrioid and clear cell carcinomas. pS2 scores were inversely linked with ER α and PR values in the nonmalignant tumor groups, with ER β values were not related to the status of any other hormone-related markers in any category.

Neither ER α nor ER β, PR, or pS2 values were linked to any of the several clinicopathological factors

| Table 1. Correlation of immunoreactivity scores for ER α, ER β, PR, and pS2 in ovarian tumors |
|---------------------------------------------------------------|-----------------|----------------|-----------------|-----------------|----------------|
|                                                              | ER α versus     |                |                | PR versus        |                |                |
|                                                              |                 | pS2 pr         |                 | PR versus        |                |                |
|                                                              |                 | r (P)           |                 | pS2 r (P)        |                 |                |
| Carcinoma                                                     |                 |                 |                 |                 |                 |                |
| Overall                                                       | 116             | 0.03 (0.78)     | 0.22 (0.02)     | 0.09 (0.34)      | 0.07 (0.45)     | 0.34 (0.0002)   | -0.21 (0.03)   |                |
| Serous                                                        | 52              | 0.17 (0.26)     | 0.004 (0.9)     | NA               | 0.25 (0.08)     | NA              |                |
| Mucinous                                                      | 18              | 0.04 (0.89)     | 0.19 (0.45)     | 0.006 (0.98)     | 0.01 (0.95)     | 0.38 (0.12)     | -0.6 (0.009)   |                |
| Endometrioid                                                  | 16              | 0.17 (0.55)     | 0.53 (0.03)     | NA               | 0.41 (0.13)     | NA              |                |
| Clear cell                                                    | 34              | 0.14 (0.44)     | 0.92 (<0.0001)  | NA               | 0.15 (0.42)     | NA              | NA              |
| LMP                                                           | 28              | 0.22 (0.28)     | 0.64 (0.0006)   | -0.74 (<0.0001)  | 0.04 (0.86)     | 0.07 (0.72)     | -0.59 (0.002)  |                |
| Adenoma                                                       | 69              | 0.04 (0.74)     | 0.49 (<0.0001)  | -0.63 (<0.0001)  | 0.04 (0.76)     | 0.07 (0.54)     | -0.52 (<0.0001)|                |

n, number of cases; r, Pearson’s correlation coefficient; NA, not applicable
Figure 3. A. Apoptotic (indicated by short arrow) and mitotic cells (indicated by long arrows) in a serous carcinoma. Original magnification, ×500. B, C. Semiserial sections of a clear cell carcinoma. Note (B) the lower immunopositivity for Ki-67 and (C) high p27Kip1 expression. Original magnification, ×200.

Figure 4. Relations between cell kinetic markers and histological tumor phenotypes. LMP, low malignant potential tumor; S, serous type; M, mucinous type; E, endometrioid type; C, clear cell type; AI, apoptotic index; MI, mitotic index; LI, labeling index. The data are mean ± SD values.
investigated for ovarian carcinomas (data not shown).

Apoptosis, cell proliferation, and p27Kip1 expression

Examples of apoptotic and mitotic cells and immunoreactivity for Ki-67 and p27Kip1 are illustrated in Figure 3. There were positive correlations between apoptosis and cell proliferation in all the categories (AI vs. MI, r = 0.52, P < 0.0001; AI vs. Ki-67 LI, r = 0.55, P < 0.0001; MI vs. Ki-67 LI, r = 0.83, P < 0.0001).

Average AI values, as well as MI and Ki-67 LIs, showed a stepwise increase from benign to malignant lesions, with statistically significant differences (P < 0.0001, respectively). In contrast, average p27Kip1 values significantly decreased with progression (P = 0.0005). In LMP and malignant tumors, these parameters showed some differences among histological phenotypes (Figure 4).

In ovarian carcinomas, significantly high MI were observed for stage III/IV vs. stage I/II lesions (P < 0.0001), positive vs. negative nodal metastasis (P = 0.011), and G3 vs. G1 tumors (P = 0.037). Similar findings were also noted for Ki-67 LIs, while there was no association with either AI or p27Kip1 LIs.

Moderately to high p27Kip1 LIs (more than the average of 59.7 ± 26.3) were significantly associated with lower MI and Ki-67 LIs (P = 0.02) but not AIs.

No relation between any of the cell kinetic markers or hormone-related molecules investigated were noted in any ovarian tumor category (data not shown).

Survival

Based on the average values for each marker, a subdivision was made into two categories to examine their relations to the survival of ovarian carcinoma patients. Figure 5 demonstrated Kaplan-Meier curves for the relations among PR status, Ki-67 LIs, and overall survival. The univariate analysis results for prognostic markers by the log-rank test revealed the prognostic values for PR score, MI and Ki-67 LI, as well as the FIGO stage and nodal metastasis (Table 2). However, the multivariate

![Figure 5. Relation between tumor-related death and (A) the immunoreactivity score for the progesterone receptor (PR) and (B) the Ki-67 labeling index (LI). The Kaplan-Meier curves show proportions of survival patients.](image-url)
analysis only showed the FIGO stage to be an independent factor for survival (Table 3).

**RT-PCR/SBH assay**

As shown in Figure 6, fragments of ERα, ERβ, PR, and pS2 genes could be coamplified with β-actin, with the exception of a few cases.

In the competitive PCR assays for both of the ER forms, ERα signals were detected in all the samples investigated, while ERβ expression was evident in 7 (25%) carcinomas, 1 (14.3%) LMP, and 6 (33.3%) cystadenomas. No differences in the relative intensity of either ERα or β were observed among the three ovarian tumor groups. Amplicons of the PR gene were identified in 19 (65.5%) malignant, 6 (85.7%) premalignant, and 17 (94.4%) benign tumors. The difference in the relative intensity was significant between cystadenomas and carcinomas (P = 0.002), in contrast to the pS2 gene, which

### Table 2. Univariate analysis of prognostic factors ovarian carcinomas

<table>
<thead>
<tr>
<th>Factor</th>
<th>Average value</th>
<th>Category</th>
<th>n</th>
<th>Log-rank c2</th>
<th>P Value</th>
<th>Favorable feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO stage</td>
<td></td>
<td>Stage I/II</td>
<td>56</td>
<td>19.64</td>
<td>&lt;0.0001</td>
<td>Stage I/II</td>
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<tr>
<td></td>
<td></td>
<td>Stage III/IV</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN metastasis</td>
<td></td>
<td>Positive</td>
<td>22</td>
<td>10.96</td>
<td>0.0009</td>
<td>No metastasis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERα score</td>
<td>1.3 ± 2.8</td>
<td>≥3</td>
<td>15</td>
<td>0.006</td>
<td>0.936</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤2</td>
<td>88</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ERβ score</td>
<td>0.7 ± 1.5</td>
<td>≥2</td>
<td>24</td>
<td>0.048</td>
<td>0.826</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤1</td>
<td>78</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PR score</td>
<td>2.5 ± 3.6</td>
<td>≥5</td>
<td>23</td>
<td>4.92</td>
<td>0.026</td>
<td>High score</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤4</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pS2 score</td>
<td>0.9 ± 2.7</td>
<td>≥1</td>
<td>13</td>
<td>1.048</td>
<td>0.305</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>88</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AI</td>
<td>0.9 ± 0.7</td>
<td>≥1</td>
<td>42</td>
<td>0.124</td>
<td>0.724</td>
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<tr>
<td></td>
<td></td>
<td>≤0.99</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>0.5 ± 0.4</td>
<td>≥0.5</td>
<td>47</td>
<td>3.97</td>
<td>0.046</td>
<td>Low index</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤0.49</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki-67 LI</td>
<td>35.7 ± 20.8</td>
<td>≥36</td>
<td>46</td>
<td>4.93</td>
<td>0.026</td>
<td>Low LI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤35.9</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p27 LI</td>
<td>59.7 ± 26.3</td>
<td>≥60</td>
<td>56</td>
<td>0.235</td>
<td>0.628</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>≤59.9</td>
<td>49</td>
<td></td>
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</table>

LN, lymph node; n, number of cases

### Table 3. Multivariate analysis of prognostic markers for ovarian carcinomas

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO stage</td>
<td>0.12</td>
<td>0.03-0.48</td>
<td>0.003</td>
</tr>
<tr>
<td>LN metastasis</td>
<td>1.71</td>
<td>0.45-6.51</td>
<td>0.43</td>
</tr>
<tr>
<td>PR score</td>
<td>0.63</td>
<td>0.16-2.56</td>
<td>0.52</td>
</tr>
<tr>
<td>MI</td>
<td>1.81</td>
<td>0.36-9.17</td>
<td>0.47</td>
</tr>
<tr>
<td>Ki-67 LI</td>
<td>0.4</td>
<td>0.08-2.12</td>
<td>0.28</td>
</tr>
</tbody>
</table>

LN, lymph node; MI, mitotic index; LI, labeling indices
Figure 6. RT-PCR/SBH results for hormone receptors and pS2. A. Competitive PCR for estrogen receptors (ER) α and ERβ with co-amplification of β-actin gene in ovarian carcinomas. B. Co-amplification of β-actin and the progesterone receptor (PR) or pS2 in premalignant (B) and cystadenoma (A) samples. Hist, histology; S, serous type; E, endometrioid type; C, clear cell type; M, mucinous type; G, grade; no, number.

Figure 7. Comparison of the mRNA expression of hormone receptor and pS2 genes among cystadenoma (Ad), LMP (Lm), and carcinoma (Ca) lesions. The significantly low level of pS2 expression in carcinomas is due to the small proportion with a mucinous phenotype as compared with that benign tumors. ER, estrogen receptor; PR, progesterone receptor. The data are mean ± SD values.
was only detected in mucinous tumors (Figure 7).

Hybridization signal intensities for PR and pS2 significantly correlated with their immunoreactivity scores (PR, $r = 0.42$, $P = 0.003$; pS2m, $r = 0.77$, $P < 0.0001$; pS2p, $r = 0.8$, $P < 0.0001$), while such associations could not be observed for $ER\alpha$ or $\beta$.

**Discussion**

Relative levels of $ER\alpha$ and $ER\beta$ mRNAs differ with tissue, the former having moderate to high expression in the uterus, testis, and ovary, and the latter in the prostate, brain, and ovary. In normal ovary, $ER\beta$ mRNA is expressed at high levels in granulosa cells of primary, secondary, and preovulatory follicles, while $ER\alpha$ is dispersed through entire tissue. A previous study demonstrated $ER\alpha$ mRNA expression to be equal or slightly higher in ovarian carcinomas than normal ovary, in contrast to the clear decrease evident for $ER\beta$ mRNA, suggesting that down-regulation of the latter may be due to a lack of any granulosa cell components in tumor tissue.

In the present study, we used a competitive PCR assay in which $ER\alpha$ and $ER\beta$ cDNA are amplified in the same reaction to investigate the relative expression of the two subtypes. To avoid-negative results, coamplification of $\beta$-actin and SHB techniques were also applied. In our series, relatively low positivity for $ER\beta$ mRNA was evident in all categories of ovarian tumors, while $ER\alpha$ mRNA was detected in all of tumor samples investigated. In contrast, Pujol et al reported $ER\beta$ mRNA to be the predominant species in normal ovary and benign ovarian tumors, while the ratios of $ER\alpha : ER\beta$ mRNAs were significantly increased in ovarian carcinomas. Although a reason for the discrepancy is unclear, the present lack of evidence for marked changes in $ER\alpha$ and $ER\beta$ mRNA expression among benign, LMP, and malignant ovarian tumors was supported by the IHC findings in the present study.

Reduction of circulating levels of estrogen by ovariectomy reduces PR concentration in PE04 ovarian carcinoma xenografts, while exogenous administration causes increase, suggesting that estrogen can regulate PR in ER-positive ovarian carcinoma cells. In the present study, a good correlation between $ER\alpha$ and PR immunoreactivity scores was observed in benign ovarian tumors, and endometrioid and clear cell carcinomas, while $ER\beta$ scores did not show any association with $ER\alpha$ or PR status. In breast carcinomas, $ER\beta$ mRNA does not correlate with $ER\alpha$ expression and is inversely associated with PR status.

The $pS2$ gene has also been considered as a member of the estrogen-inducible gene family, because estrogenic stimulation can increase $pS2$ expression at transcription levels in breast cancer cells. In the present series, however, $pS2$ expression in terms of both mRNA and protein was limited to mucinous ovarian tumors, and it appeared to be inversely associated with $ER\alpha$ and PR status. Considering abundant $pS2$ found in gastric mucosa lacking $ER$ expression, its expression may simply be associated with the mucin-production feature in ovarian tumors, independent of the hormone status.

Cell deletion and proliferation is tightly regulated by synthesis and activation of a number of positive and negative regulators of cell cycle progression. Overexpression of $p27Kip1$ in mammalian cells induces a G1 block of a cell cycle progression and inhibits cell growth, while its overexpression can also promote apoptosis in a variety of human malignancies. Our current data suggested that AIs showed a stepwise increase from benign to malignant ovarian tumors, and were positively associated with cell proliferation, in contrast to decreased $p27Kip1$ expression. Although we could not demonstrate any significant link between AIs and $p27Kip1$ status, this was in accordance with the role of $p27Kip1$ as a negative regulator of the cell cycle.

Univariate analysis revealed moderate to high levels of PR expression to be significantly associated with a favorable outcome in ovarian carcinoma patients, as well as low MI and Ki-67 LIs. Similar findings have also been reported by other investigators. Considering the evidence that progesterone can inhibit cell proliferation through induction of cell differentiation in endometrial and ovarian carcinomas, a possible role of PR in the regulation of tumor kinetics might be expected. These results, however, revealed that PR expression, as well as $ER\alpha$ and $ER\beta$ status, was not related to any cell kinetic markers investigated. We therefore concluded from the present data that hormone-related pathways may not be directly linked with the growth of ovarian carcinomas. This observation may be in agreement with the failure of ER and PR status to predict a response to hormone therapy.

The lack of association between IHC and mRNA results in PR expression, in particular of the $ER\alpha$ subtype, was of interest in the present study, in contrast to PR and pS2 analyses, which show significant correlations. Possible reasons for these anomalous results include the following: 1. the presence of stromal cells expressing ER and 2. the high sensitivity of PCR- compared with a protein-based assay. However, Brandenberger et al have also proposed that ER mRNA levels may not always
correlate with protein levels, and therefore may not indicate biologically active ERs.\textsuperscript{12}

In conclusion, the present study demonstrated that ER\textalpha rather than ER\textbeta may play an important role in the regulation of PR, but not pS2 expression in ovarian tumors. Moreover, independent of changes in tumor tissue kinetics, PR expression significantly indicates a good prognosis for women with ovarian carcinomas.

References


