Expression of human estrogen receptor-α and -β, progesterone receptor, and androgen receptor mRNA in normal and malignant ovarian epithelial cells

KIN-MANG LAU*, SAMUEL C. MOK†, AND SHUK-MEI HO*‡

*Department of Biology, Tufts University, Medford, MA 02155; and †Laboratory of Gynecological Oncology, Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02115

Communicated by Elwood V. Jensen, Karolinska Institute, Huddinge, Sweden, March 15, 1999 (received for review September 15, 1998)

ABSTRACT Our understanding of the roles played by sex hormones in ovarian carcinogenesis has been limited by a lack of data concerning the mode of sex hormone action in human ovarian surface epithelial (HOSE) cells, the tissue of origin of >90% of ovarian cancers. We have compared the relative abundance of estrogen receptor (ERα), ERβ, progesterone receptor (PR), and androgen receptor (AR) mRNA in four primary cultures of HOSE cells obtained from postmenopausal women to those found in late serous adenocarcinoma primary cell cultures and established ovarian cancer cell lines. We observed coexpression of ERα and ERβ mRNA along with AR and PR transcripts in normal HOSE cells and disruption of ERα mRNA expression as well as dramatic down-regulation of PR and AR transcript expression in most ovarian cancer cells. In contrast, levels of ERβ mRNA were unaffected by the malignant state. Additionally, a novel mutation involving a 32-bp deletion in exon 1 of ERα transcripts was detected in the SKOV3 cell line. This mutation would explain why SKOV3 was reported to be ER-positive but estrogen-insensitive. Taken together, these findings suggest that estrogens, signaling via either or both ER subtypes, may play an indispensable role in regulating normal HOSE cell functions. Therefore, loss of ERα, PR, and AR mRNA expression in HOSE cells may be responsible for neoplastic transformation in this cell type. In contrast, the roles played by ERβ in normal and malignant HOSE cells remain elusive. Finally, the coexistence of mutated ERα mRNA and normal ERβ transcripts in SKOV3 argues in favor of a dependency of ERβ action on functional ERαs.

Ovarian carcinoma (OC) is the second most common and the most deadly malignancy of the female reproductive tract (for review, see refs. 1–4). Etiological factors involved in ovarian carcinogenesis remain poorly defined, and effective treatment protocols are limited (1–3). Epidemiological data suggest that endogenous and exogenous sex hormones may play important roles in the pathogenesis of the disease. In this regard, estrogens taken as oral contraceptives during premenopausal years offer protection, but when used postmenopausally as hormone replacement therapies elevate risk (1–6). The risk of developing invasive OC increases with ever-use of hormone replacement therapy and has been shown to depend on the duration of usage (5, 6). In addition to estrogens, other ovarian or adrenal steroids such as androstenedione, testosterone, and progesterone have all been implicated as risk factors for OC (1–4). Androstenedione and progesterone are present at higher concentrations in the ovarian vein draining the affected ovary when compared with levels found in the vein draining the contralateral, disease-free gland (7, 8). Plasma levels of estradiol-17β (E2), progesterone, 20α-hydroxyprogesterone, testosterone, and androstenedione have all been shown to correlate with OC tumor masses (9–11). Taken together, these findings suggest that steroid hormones are likely involved in the genesis and progression of the disease, yet their mechanisms of action remain unclear.

The classical estrogen receptor (ER), recently renamed ERα (12), and the progesterone receptor (PR) were found in <50% of OC specimens, whereas androgen receptor (AR) was detected in most cases (>80%) (1–4). In recent studies, transcripts of the newly discovered ER subtype, ERβ (12), were found in normal human ovaries and benign and malignant ovarian tumors (13, 14), as well as in primary cultures of normal human ovarian surface epithelial (HOSE) cells (15). Unfortunately, none of the aforementioned studies demonstrated a strong association between ERα, PR, or AR status and OC histological types or grades. Furthermore, treatments of OCs with tamoxifen, antiandrogens, or progestins produced very dismal responses (for review, see refs. 1–4). Consequently, it is widely believed that levels of sex hormone receptors have little prognostic value and are poor predictors of hormone-manipulation outcomes for OCs.

A major challenge in assessing the significance of sex hormones and their receptors in ovarian carcinogenesis is the paucity of information about their expression levels in the normal HOSE. Over 90% of OCs arise from the HOSE, which shares a common embryonic origin with epithelia of Mullerian duct-derived tissues (Fallopian tube, endometrium, and endocervix) but is distinctly different from the granulosa–thecal cells of the ovary (1–4). In terms of tissue mass, this layer represents only a small fraction of the whole ovary. Thus, data generated from studies that compare levels of a molecular marker found in OCs with those observed in whole ovaries are difficult to interpret because expression pattern in HOSE could easily be masked by those in other ovarian cell types. In this regard, although previous studies have demonstrated localization of ERβ in ovarian granulosa cells and ERα throughout the ovary (12, 16, 17), only recently have both ER subtypes been found in normal HOSE cells (15). However, it remains unclear as to whether their expression levels are altered after neoplastic transformation. Importantly, little is known about the relationships between the expression patterns of the two ER subtypes and those of other steroid receptors in normal and malignant HOSE cells.

Abbreviations: HOSE, human ovarian surface epithelium; ER, estrogen receptor; PR, progesterone receptor; AR androgen receptor; OC, ovarian carcinoma; RT-PCR, reverse transcription–PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

To whom reprint requests should be addressed: Department of Surgery, Division of Urology, University Campus, University of Massachusetts Memorial Medical Center, 55 Lake Avenue North, Worcester, MA 01655.
To fill this data gap, in the present study, we have examined the expression of ERα, ERβ, PR, and AR transcripts in primary cultures of normal HOSE cells, obtained from postmenopausal women, to those found in primary ovarian cancer cell lines and established OC cell lines. This approach has allowed us to observe that (i) ERα and ERβ mRNA, as well as AR and PR transcripts, are coexpressed in normal HOSE cells, (ii) whole exon-deletion variants of ERα and ERβ are commonly found in HOSE and ovarian cancer cells, and (iii) PR and AR mRNA expression is significantly down-regulated in ovarian cancer cells that exhibit altered ERα, but normal ERβ, message expression. A previously unknown ERα mutation, leading to a 32-bp deletion in exon 1, has been identified in an ovarian cancer cell line, SKOV3. This cell line has been shown to express ERα but is estrogen- and antiestrogen-resistant (18). Taken together, these findings implicate regulation of normal HOSE cell functions by estrogens, and possibly by progestins and androgens. Additionally, the emergence of sex hormone resistance, via down-regulation or mutational inactivation of receptors, may be a key feature of ovarian epithelial transformation.

MATERIALS AND METHODS

Cell Cultures and Cell Lines. Four primary cultures of normal HOSE cells (HOSE27, HOSE20, HOSE17, and HOSE13), one primary culture of normal mesothelial cells (MesO13), four primary cultures of ovarian carcinoma cells (OVCA420, OVCA429, OVCA432, and OVCA433), and three ovarian cancer cell lines (DOV13, SKOV3, and CAOV3) were used in this study. Primary cultures of normal HOSE cells were initiated from surface scrapings of normal ovaries removed from postmenopausal women with benign gynecological diseases according to Tsao et al. (19). In each case, ovarian histology was performed by a pathologist, and only normal ovaries were used for normal HOSE cell collection. The one primary culture of normal mesothelial cells (MesO13) was obtained from peritoneal washing of a non-OC patient. Primary cultures of ovarian cancer cells (OVCA420, OVCA429, OVCA432, and OVCA433) were established from freshly isolated ascites or tumor explants obtained from patients with late stage serous ovarian adenocarcinomas according to Tsao et al. (19). All normal HOSE and ovarian cancer primary cell cultures were early passages in medium 199 and MCDB 105 (1:1) (Sigma) supplemented with 10% fetal calf serum (Sigma), 100 units/ml penicillin, and 100 μg/ml streptomycin under 5% CO2. Normal and malignant cells grew in this medium after b, amounts of CA125 (ovarian epithelial transformation. Possibly by progestins and androgens. Additionally, the emergence of sex hormone resistance, via down-regulation or mutational inactivation of receptors, may be a key feature of ovarian epithelial transformation.

RESULTS

ERα mRNA: Expression Levels and Identification of Exon 2, 4, 5, and 7 Deletion Variants. By using primer set 1 (Table 1), which amplified a 650-bp PCR product corresponding to nucleotides 41–690 (exons 1–3) of the ERα mRNA, we demonstrated expression of ERα transcript in all primary cultures of normal HOSE cells (HOSE27, HOSE20, HOSE17, and HOSE13) and in a primary culture of mesothelial cells (MesO13) (Fig. 1). Among the primary cultures of ovarian cancer cells and the established OC lines, six of seven expressed ERα mRNA. The levels of this transcript in the three established cancer cell lines (CAOV3, DOV13, and SKOV3) were comparable to those found in HOSE cell cultures. However, ERα mRNA expression in primary cultures of ovarian cancer cells (OVCA433, OVCA432, OVCA429, and OVCA420) exhibited marked variability. Down-regulation of expression was noted in OVCA433 and OVCA420, enhanced...
expression was noted in OVCA429, and close-to-normal expression was noted in OVCA429.

In addition to the predicted 650-bp PCR product, PCR with primer set 1 amplified an additional product of a smaller size. This variant product was present in all normal and some malignant ovarian cell lines or cultures (note the fainter band in HOSEs, MesO13, and OVCA429 in Fig. 1A). After excision, reamplification, and direct sequencing, it became apparent that this smaller size PCR product represented an ER\(\alpha\) mRNA variant deleted in exon 2 (ER-\(\alpha\)Δ2, Fig. 1A and B; Fig. 2Right). Further analysis showed additional ER\(\alpha\) variants with exon 4, 5, or 7 deletion in cellular RNA samples isolated from all HOSE cell cultures and some cancerous ovarian epithelial cells (data not shown).

**Identification of an ER\(\alpha\) Exon 1 Deletion Mutant in SKOV3.** On closer examination, it was noted that PCR products derived from amplification of SKOV3 cellular RNA, using primer set 1, were of smaller than expected sizes (Fig. 1B). Subsequent DNA sequencing of these PCR products revealed the presence of a 32-bp deletion in exon 1 in both (ER\(\alpha\)-Mut and ER\(\alpha\)-MutΔ2, Fig. 1B and Fig. 3Right). No normal ER\(\alpha\) mRNAs were present in SKOV3 cells based on our analyses. Sequence analyses predicted a frameshift resulting in the production of a truncated ER\(\alpha\) polypeptide of 145 aa (containing a partial A/B domain) from the mutated ER\(\alpha\) transcript.

**ER\(\beta\) mRNA Expression.** ER\(\beta\) mRNA expression was detected in all normal and malignant ovarian cell primary cultures/cell lines examined (PCRs were performed with primer pair Set 1, Fig. 1A). No dramatic differences in mRNA expression levels were observed among primary cultures of normal HOSE cells and ovarian cancer primary cell cultures/cell lines. A slight up-regulation of ER\(\beta\) mRNA expression (≈2-fold of HOSE cells’ levels) was detected in SKOV3 and CAOV3, two established cancer cell lines. In light of a recent report of a ER\(\beta\) exon 5 deletion variant (20), we used primer set 2 (Table 1) to demonstrate the presence of this variant in all normal HOSE cell cultures and in some ovarian cancer cell lines/cultures (data not shown).

**PR mRNA Expression.** Expression of PR mRNA was demonstrated in all normal ovarian epithelial cells including the four primary cultures of normal HOSE cells and the one culture of mesoepithelial cells, MesO13 (Fig. 1, data from a 30-cycle PCR). PR mRNA expression in these cell cultures was strong. In contrast, most of the ovarian cancer cell lines/primary cultures (5/7) failed to express PR transcripts (negative expression was confirmed with 40-cycle PCRs, data not shown). Two ovarian cancer cell lines/cultures, OVCA432 and CAOV3, expressed extremely low levels of the transcript (<1/20 of HOSE cells’ levels).

**AR mRNA Expression.** Thirty-cycle RT-PCR analyses revealed the presence of AR transcripts in all HOSE cell cultures and in the MesO13 culture (Fig. 1). Fairly uniform signal intensities were observed in these samples. Of the seven ovarian cancer cell lines/primary cultures, five did not express the AR transcript (40-cycle RT-PCR was used to confirm negativity, data not shown). Nevertheless, low levels of AR mRNA expression (<1/3 of HOSE cells’ levels) were found in OVCA420 and OVCA432.

**DISCUSSION**

In this study, semiquantitative RT-PCR and direct sequencing were used to investigate ER\(\alpha\), ER\(\beta\), PR, and AR mRNA expression in normal and malignant ovarian epithelial cells. Of significance to ovarian carcinogenesis, we report (i) coexpression of ER\(\alpha\) and ER\(\beta\) wild-type transcripts and their variants in four primary cultures of HOSE cells, the tissue of origin of >90% of OCs, and in one mesothelial cell culture (ii) loss of ER\(\alpha\), but not ER\(\beta\), mRNA expression in primary cultures of ovarian cancer cells and established OC cell lines, and (iii) expression of PR and AR mRNA in normal HOSE cells and marked down-regulation of these messages in ovarian cancer cells. Additionally, a novel ER\(\alpha\) mRNA mutant, with a 32-bp deletion in exon 1, was identified in the estrogen-resistant
ovarian cancer cell line, SKOV3 (18). Finally, several ER mRNA variants, commonly found in breast cancer specimens, were detected in HOSE cells as well as in ovarian cancer cells. These variants include ERα mRNA variants that lack exon 2, 4, 5, or 7, as well as an ERβ mRNA variant deleted in exon 5.

Our finding that ERα and ERβ transcripts are uniformly expressed in HOSE cells suggests that estrogens, via ER signaling, may play an important role in regulating normal HOSE cell functions. Because PR is a well recognized estrogen-regulated gene (23, 24) and high levels of PR mRNA are found in HOSE cells, we speculate that one or both ER isoforms are functional in these cells. Our data are in agreement with recent findings (15) that demonstrated expression of ERα and ERβ in 21-day cultured HOSE cells established from fresh ovarian scraping. However, when Brandenberger and coworkers (16) evaluated ERα and ERβ mRNA expression in normal human ovaries and in one immortalized HOSE cell line (IOSE-Van; ref. 25), they detected that ERβ as the predominant ER isoform in normal human ovary but found only ERα mRNA expression in IOSE-Van. Data from this latter report cannot be used for direct comparison with our findings because human ovaries are composed of many cell types, including granulosa cells, which express high levels of ERβ (12, 16, 17). Furthermore, IOSE-Van is a HOSE cell line immortalized by SV40 and therefore may exhibit receptor characteristics different from those found in normal HOSE cells.

Of particular interest to normal HOSE cell physiology is our observation that HOSE cells derived from postmenopausal women expressed substantial levels of ERα, ERβ, PR, and AR transcripts. These findings suggest that sex hormone responsiveness is retained in HOSE cells after menopause. It is well

FIG. 3. Sequence analyses of PCR products of ERα cDNA derived from SKOV3 mRNA as compared with that derived from HOSE17 mRNA. A 32-bp deletion in exon 1 of the transcript was detected in transcripts of SKOV3 cells.
established that circulating estrogen levels are low after meno-
pause but that the ovary continues to produce androgens and estra
genams via aromatization (26, 27). Hence, ovarian estrogens
and/or androgens may be responsible for maintained normal
HOSE cell functions after menopause. In this connection, a
logical question is whether loss of sex hormone regulation in
HOSE cells is involved in the development of OCs that
dramatically increase in incidence in women after the age of
45 (1–4).

Compared with HOSE cells, ovarian cancer cells express
reduced levels of AR mRNA and no PR transcripts. Parado-
Xically, the loss of PR message expression in these malignant
cells is not attended with concomitant down-regulation of ERα
or ERβ mRNA expression. ERα mRNA expression is absent
in only two ovarian cancer cell cultures (OVCA433 and
OVCA420), whereas ERβ messages are present in all cancer
cell cultures/lines at levels comparable to those found in HOSE
cells. A lack of PR expression in ERα/ERβ-positive ovarian

cancer cells may represent a state of estrogen resistance
because, in most other estrogen-target tissues, PR expres-
sion is tightly associated with ERα (23, 24) or ERβ (28, 29)
expression. The notion that ovarian cancer cells are estrogen-
resistant is supported by clinical observations that tamoxifen
treatment is a not an effective therapy for OCs and only 36%
of OC specimens coexpress both ERα and PR (1–4).

The mechanisms underlying estrogen resistance have not
been investigated in OCs. However, results from breast and
endometrial cancer studies have identified reduction in ER
expression, presence of dominant-negative ER variants, mu-
tational inactivation of ER, and abnormal expression of co-
activators/repressors of ER as probable causes for this phe-

nomenon (30–34). ERα mutations are rare but have been
reported in metastatic and tamoxifen-resistant malignancies of
the breast (32, 33) and of the endometrium (34). Nevertheless,
ERα mutations have not been reported in ovarian cancer
specimens. In the present study, by using primers designed to
amplify exon 1–3 of the ERα, we discovered an ERα mRNA
mutation with a 32-bp deletion in exon 1 in SKOV3 cells.
Based on sequence analyses, this deletion was predicted to
cause a frameshift and result in the synthesis of a truncated,
145 aa protein. This protein, if synthesized in vivo, would lack
both the DNA- and ligand-binding domains and likely be
inactive. Previously, Hua and coworkers had reported SKOV3
cells to be estrogen-nonresponsive (18). These cells were
shown to be insensitive to estrogen with respect to cell
proliferation and induction of gene expression, and yet, they
expressed ERα at both message and protein levels. Their latter
findings do not contradict our results because, in their study,
ERα messages were analyzed by using Northern hybridization,
a method apt to miss a 32-bp mutation. Additionally, in their
Western blot analyses, the mAb D75, instead of the ERα
specific H222 (35), was used. Hence, cross-reactivity with ERβ
might have been a confounding factor. Our result clearly
demonstrated expression of ERβ mRNA in SKOV3 cells, and
if proteins were synthesized, immunoreactivity with D75 is
conceivable. According to contemporary views (for review see
ref. 36), if ERα and ERβ are coexpressed in the same cell, they
may regulate different sets of cellular functions or play re
dundant roles. In the case of SKOV3 cells, mutated ERαs and
wild-type ERβs likely coexist. It is therefore possible that
estrogen resistance in this ovarian cancer cell line is because of
mutational inactivation of the ERα. What remains to be
explained is the roles played by ERβ in mediating estrogen
responsiveness in SKOV3 cells, because this cell line expresses
normal levels of ERβ mRNA. A comparable situation exists in
ovarian granulosa cells of ERKO (ERα-knockout) mice (37).
Disruption in folliculogenesis and abnormal granulosa cell
functioning have been observed in these animals, and yet high
levels of ERβ mRNA, but no ERα, are present in their
granulosa cells (37). These findings in ERKO mice, together
with our data, on SKOV3 suggest that the action of ERβ may
depend on the presence of a functional ERα. If this supposition
is verified, it would have significant implications in ERα/ERβ
action.

ER mRNA variants are frequently expressed in many normal
and malignant tissues (for review, see ref. 30). In breast
cancers, variants of ERα are prevalent and they almost always
coeexist with wild-type ER (30). Even though little evidence has
been presented in support of actual translation of these mRNA
variants to protein products, in vitro studies suggest that they
may have “outlaw functions,” i.e., dominant-positive or dom-
ninant-negative functions (30). Previously, one ERα variant
with exon 4 deletion has been reported in OC specimens and
in normal human ovaries (38). Our data now demonstrates
prevalent occurrence of ER mRNA variants, coexisting with
wild-type messages, in HOSE and ovarian cancer cells. Spe-
cifically, ERα mRNA variants that lack exon 2, 4, 5, or 7, as well
as an ERβ mRNA variant deleted in exon 5 (20) were detected.
Because the presence of ER mRNA variants is in both normal
and malignant HOSE cells, this finding implies that mecha-
nisms underpinning ER mRNA variant generation remain unaltered following neoplastic transformation.

Until recently, the biological significance of progesterone or
androgen in ovarian carcinogenesis has remained unknown.
Loss of estrogen responsiveness in HOSE cells likely leads to
down-regulation of PR. Progesterone or progesterone respon-
siveness appears to offer protection against ovarian carcino-
genesis. Previous epidemiological studies reported a reduction
in OC risk in postmenopausal women using the combination
(estrogens plus progestins) hormone replacement therapy (4,
39). In a recent epidemiological study, an increase in ovarian
cancer incidence was observed among women with progester-
one deficiency (40). In another study, 93% of malignant
ovarian tumors exhibited PR immunonegativity, whereas PR
immunopositivity was observed in the majority of borderline
tumors (41). Exposure of ovarian cancer cells to progesterone
up-regulated p53 and induced apoptosis in ovarian cancer cell
lines (42). Similarly, testosterone and androstenedione were
found to be effective in suppressing ovarian cancer cell pro-
liferation (43). Of interest, a 75% rate of loss of heterozygosity
at 11q23.3–24.3 that harbors the PR gene locus (44, 45) had
been reported, and one of us (S.C.M.) recently detected a 40%
loss of heterozygosity at Xq11.2–q12 that harbors the AR gene
(46). In the present study, we observed significant down-
regulation of PR and AR mRNA expression in several estab-
lished ovarian cancer cell lines and in the small number of
primary cell cultures established from patients with late serious
adenocarcinoma. Taken together, these findings suggest that
progesterone and testosterone signaling, via their cognate
receptors, may have tumor suppressor function in ovarian
carcinogenesis by induction of apoptosis and/or inhibition of
proliferation. Hence, diminution in progesterone and/or an-
drogen action/response may predispose HOSE cells to neo-
plastic transformation.

In summary, by comparing receptor mRNA levels in ovarian
cancer cells to those found in HOSE cells, we have noted
disruptions of ERα, PR, and AR mRNA expression in cancer
cells. The association between loss of PR and/or AR expres-
sion and malignancy is especially apparent. It is conceivable
that progesterone and/or androgen actions may protect HOSE
cells from ovarian cancer transformation. If this premise can be
verified, it may have significant clinical implications in post-
menopausal hormone replacement therapy management. In
contrast, expression of ERβ mRNA and those of several forms
of ER mRNA variants are not affected by the malignant state.
Future experiments are necessary to reveal the biological
significance of these molecules in both normal HOSE cell
physiology and ovarian tumorigenesis. Finally, of particular
interest, the newly discovered ERα mutation involving a 32-bp
deletion in exon 1 of the transcript in SKOV3 cells may explain
the previously observed estrogen resistance in this ovarian cancer cell line.

This research is supported in part by National Institutes of Health—National Cancer Institute Grants CA-15776, CA62269, and AG13965 to S.M.H. and CA60453 and CA69291 to S.C.M.