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A useful cell system for studying the regulation of 17HSD/KSR type 2 activity and expression in ovarian epithelial cancer

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This paper is dedicated to the memory of Paul H. Lima and of Dr. Charles H Blomquist.

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ABSTRACT

17β-Hydroxysteroid dehydrogenase/17-ketosteroid reductase (17HSD/KSR) activity and 17HSD/KSR types 1, 2, 4, and 5 mRNA levels were characterized in ovarian cancer cell lines derived from patients unexposed to radiation or chemotherapy. Activity was at the limit of detection in TOV-112D and TOV-21G cells. Activity in OV-90 was comparable to that in human placental tissue, was predominantly microsomal and was 17HSD/KSR type 2-like in substrate specificity and inhibition patterns. In monolayers, conversion of testosterone (T) to androstenedione (A) was 12-fold greater than that of A to T. Reduction of fetal bovine serum to 0.3% in the culture medium had no effect on 17β-HSD activity. Significant levels of type 1 and type 2 mRNAs were observed in OV-90 while only trace amounts were detected in TOV-21G. In contrast, type 4 mRNA levels were comparable for OV-90 and TOV-21G. Type 5 mRNA was detected in both cell lines but its level in OV-90 was twice that of TOV-21G. In OV-90, the type 2-like activity was predominant even though the type 5 mRNA level was 2.5-fold higher than that of the type 2. OV-90 cells may be a useful system for studying the regulation of 17HSD/KSR type 2 activity and expression in ovarian epithelial cancer.

1. Introduction

Epithelial ovarian cancer is the most lethal of the common gynecologic malignancies [1]. Although sex steroids have been implicated in the pathogenesis and growth regulation of ovarian carcinomas, endocrine based therapies have yielded mixed results [2–4]. Because a large percentage of epithelial ovarian cancer patients are post-menopausal, intra-tumoral formation and metabolism of estradiol (E_2), in particular, and testosterone (T) are of interest. 17β-Hydroxysteroid dehydrogenases/17-ketosteroid reductases (17HSD/KSR) catalyze pyridine nucleotide-dependent oxidation and reduction at the C-17 position of C_{18}- and C_{19}-17β-hydroxysteroids and 17-ketosteroids. Because the presence of a 17β-hydroxyl group markedly affects the binding affinity of these steroids for receptor, 17HSD/KSRs are currently thought to regulate receptor occupancy not only in normal human tissues but also in tumors [5]. A number of isoforms of 17HSD/KSR have been characterized [6,7]. Although they are termed isoforms, it is important to note that these are products of separate genes and differ in their subcellular localization, steroid substrate and pyridine nucleotide cofactor specificity and relative oxidative or reductive activity under non-equilibrium conditions in intact cells. Some isoforms also have 3α-HSD/3-KSR or 20α-HSD activity [8]. Because ovarian epithelial tumors contain tumor cells and stromal elements, the extent to which tumor cells *per se* contribute to the regulation of steroid action is of fundamental interest not only for our understanding of tumor pathophysiology but also as a basis for estimating the therapeutic potential of enzyme-specific inhibitors.

Ovarian epithelial tumors are derived from ovarian surface epithelium and its inclusion cysts [9,10], and there is evidence of a role for androgens, estrogens and progesterone in tumor pathophysiology [11–13]. Normal surface epithelial cells in culture, as well as benign and malignant epithelial tumors contain estrogen, progesterone and androgen receptors (cf. [14] and references therein). Relatively abundant expression of mRNA for various steroidogenic enzymes including members of the steroid dehydrogenase/reductase superfamily was reported for human ovarian surface epithelial cells [15]. Both benign and malignant tumors have also been shown to produce androgens and estrogens suggestive of autocrine or paracrine relationships between steroidogenesis, steroid metabolism and receptor function [16].

Recent studies have demonstrated mRNA expression for 17HSD/KSR types 1, 2, 4 and 7 in cultures of normal surface epithelium [15,17]. Our group has presented kinetic evidence of an androgenic form of 17HSD/KSR in the CAOV-3 and OVCAR-3 cell

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Table 1
17HSD/KSR activity in three different ovarian cancer cell lines.

Cell line	Origin	E_2 to E_1 (pmol/mg protein/30 min)	T to A (pmol/mg protein/30 min)
OV-90	Malignant ascites serous adenocarcinoma	8754 ± 220	12,537 ± 61
TOV-21G	Clear cell carcinoma grade 3	21.1 ± 0.9	19.0 ± 0.1
TOV-112D	Endometrioid carcinoma	26.2 ± 3.2	34.4 ± 7.5

Triplicate cultures were combined, sonicated and assayed in duplicate at pH 9.0 with 0.5 mM NAD and 1.0 μM [^{3}H]-E_2 or [^{3}H]-T. The values are the mean ± SD of duplicate assays. Estradiol (E2), estrone (E1), testosterone (T), androstenedione (A).

lines derived from an ovarian adenocarcinoma but there was no evidence of estrogenic type 1 isoform [18]. In addition, activity in these cell lines was low when compared with levels we subsequently observed in samples of epithelial tumor tissue [19,20] suggestive of the possibility that activity may be lost in cell lines established from primary tumors. As has been pointed out, one possible confounding factor may be that many of the current cell lines were established from patients exposed to radiation and or chemotherapy [21]. As an approach to resolving this latter issue, the study reported here was undertaken to characterize 17HSD/KSR activity in cytosol and microsomes as well as monolayer cultures, and mRNA expression in cell lines established from patients who had never been exposed to either radiation or chemotherapy [21,22]. Preliminary activity measurements carried out under conditions shown to be useful in detecting various isoforms in other cells and tissues [23] suggested that 17HSD/KSR types 2 and 4 were present at a high level in one of the cell lines. The current study was undertaken to clarify the nature of the predominant isoform and the relationship between activity patterns and mRNA expression levels.

2. Materials and methods

2.1. Cell culture

Human cell lines were obtained from the American Type Culture Collection. Except where indicated cells were cultured in DMEM/F-12 medium supplemented to 10% with fetal bovine serum. OV-90, TOV-21G and TOV-112D are ovarian adenocarcinoma cells with an epithelial morphology. For more details, see Table 1.

2.2. Cell fractionation

Cells were collected from monolayers by scraping followed by centrifugation at 1000 × *g* for 10 min. Cell pellets were suspended and homogenized in buffer containing 0.04 M potassium phosphate, pH 7.0, 1.0 mM EDTA and 20% (v/v) glycerol (GKP) and homogenized by hand in a glass Dounce homogenizer with an A pestel. Homogenates were centrifuged at 1000 × *g* for 10 min to remove cell debris and then 105,000 × *g* for 60 min to obtain cytosol and microsomes. The particulate fraction enriched in microsomes was suspended in GKP to a volume equal to that of the cytosol and along with cytosol stored at 4 °C.

2.3. Enzyme activity assays

17HSD/KSR and 3α-HSD/KSR activities were assayed as described previously [19,24]. Briefly, 10-μl aliquots of sample were combined with 10 μl of reaction mixture to give a 20-μl reaction mixture containing 1.0 μM ^{3}H-labeled steroid substrate, 0.5 mM pyridine nucleotide cofactor and 0.04 M buffer. Steroid substrates and inhibitors were added in methanol to a final concentration of 2% (v/v). Substrate and product were separated by thin layer chromatography and the percent conversion of substrate into product converted into specific activities (pmol/mg protein) as previously reported [18]. Further details for particular assays are given in the figure legends.

2.4. mRNA preparation and cDNA probes

Total RNA was prepared from 3×10^7 cells by lysis in 5.0 ml of Tri Reagent, a mixture of phenol and guanidine thiocyanate in a monophasic solution (Molecular Research Center, Cincinnati, OH, USA). RNA was separated from DNA and proteins by the addition of 200 μl of chloroform per ml of Tri Reagent used. RNA was recovered by precipitation with 1.2 ml of isopropanol. RNA (15 μg) was glyoxalized, resolved by 1.2% (w/v) agarose gel electrophoresis, transferred to Nytran$^+$ membrane (Schleicher & Schuell, Inc., VWR) [25], and prehybridized, hybridized and washed under high stringency conditions [26]. Probes were 17HSD/KSR type 2 (1.3 kb) [27] and γ-actin (2 kb) [28] full-length fragments, and the EcoRI/EcoRI 1.4 kb fragment of 17HSD/KSR type 4 [29]. They were labeled with (α^{32}P)deoxy-CTP to 2×10^6 dpm/ng with random primers [30].

2.5. Quantitative PCR

After extraction, RNA was purified on a CsCl gradient as described [25]. cDNA synthesis and real-time quantitative PCR were performed as described previously [31]. Briefly, an aliquot of 4 μg of total RNA was treated with DNase I (0.25 unit/μg of total RNA), and reversed transcribed (Superscript II, Invitrogen) according to the protocol of the manufacturer, using hexameric random primer (pd(N)6, Invitrogen) in a final volume of 20 μl. LightCycler-FastStart DNA Master SYBR Green I kits (Roche) were used for real-time PCR. Reactions were performed according to the protocol of the manufacturer with 0.5 μM of each primer (final concentration), 3 mM $MgCl_2$, and an amount of cDNA samples corresponding to 100 ng of total RNA input in a final volume of 20 μl. After enzyme activation (10 min, 95 °C), PCR cycles were performed: 5 s, denaturation 95 °C; 5 s, annealing temperature (see below); 20 s, elongation 72 °C; 5 s, temperature of fluorescence intensity reading (see below). At the end of each run, samples were heated to 95 °C with a temperature transition rate of 0.2 °C/s to construct dissociation curves. Several PCR reactions were tested by gel electrophoresis, and amplicons were subjected to DNA sequencing to confirm specificity of the reactions. A standard curve was prepared for each gene using a specific amplicon previously obtained by PCR. The program supplied by the manufacturer (LightCycler Software, Version 3.5) was used to import the standard curves and calculate the amount of PCR products. Normalization factors were calculated using the geNorm program [32] and QPCR data obtained for YWHAZ (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide), TBP (TATA box binding protein), HPRT1 (hypoxanthine phosphoribosyltransferase 1), and SDHA (succinate dehydrogenase complex, subunit A, flavoprotein) housekeeping genes. Other information on QPCR conditions including 5′ oligonucleotide, 3′ oligonucleotide, length of amplicon, annealing temperature, and temperature of fluorescence intensity reading for HSD17B1 (17HSD/KSR type 1), HSD17B2 (17HSD/KSR type 2), HSD17B5 (17HSD/KSR type 5), YWHAZ, TBP, HPRT1, and SDHA were previously reported [20]. The amount of mRNA molecules per 100 ng total RNA input was calculated from the amount of specific cDNA template (nanograms) obtained by

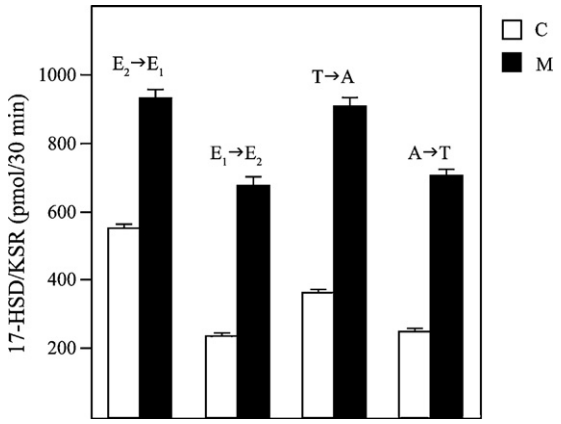

Fig. 1. 17HSD/KSR activity in OV-90 cell fractions. 17HSD/KSR activity was assayed with estradiol (E_2), estrone (E1), testosterone (T) and androstenedione (A) in cytosol (C) and microsomes (M) from approximately 3×10^6 OV-90 cells. Dehydrogenase assays contained 0.5 mM NAD^+ and 1.0 μM [^{3}H]-E_2 or [^{3}H]-T in 0.04 M Bicine, pH 9.0. Reductase assays contained 0.5 mM NADH and 1.0 μM [^{3}H]-E_1 or [^{3}H]-A in 0.04 M Hepes (pH 7.2). The values are the mean ± SD of duplicate assays.

real-time PCR and the molecular weight of each double-stranded specific cDNA sequence.

2.6. Western blot analysis

Western blot analysis was performed as described [33]. The following antibodies were used: an anti-human HSD/KSR type 2 [33] and an anti-human HSD/KSR type 5 (clone NP6.G6.A6, Sigma; kindly provided by Dr. T Penning, University of Pennsylvania, USA) [34].

3. Results

3.1. Enzymatic activities

When sonicates were assayed under optimal conditions for detecting 17HSD/KSR activity with E_2 and T, OV-90 cells had a high level of activity whereas activity was at the limit of detection in TOV-21G and TOV-112D cells (Table 1). The level of activity in OV-90 cell sonicates was comparable to that in human placental microsomes [23] and in ovarian mucinous cystadenomas [19]. An E_2/T activity ratio of 0.7 was suggestive of a prominent contribution of 17HSD/KSR type 2 to activity.

17HSD/KSR type 2 is a microsomal enzyme equally reactive with both E_2 and T as substrates [23,35]. As shown in Fig. 1, when cells were fractionated into cytosol and microsomes, activity was detected in both subcellular fractions; the highest activity with each substrate was associated with microsomes. Dehydrogenase activity exceeded reductase activity with each substrate.

The E_2/T activity ratio of approximately 1.0 was consistent with the presence of 17HSD/KSR type 2. As an approach to confirming this, T was tested as an inhibitor of the conversion of E_2 to E_1 and E_2 was tested as an inhibitor of the conversion of T to androst-4-ene-3,17-dione (androstenedione, A). Inhibition patterns for cytosolic and microsomal activities were nearly identical and consistent with E_2 and T interacting at a common site (Fig. 2). However in each case, 10–30% of activity was not inhibited.

As a further approach to confirming the presence of 17HSD/KSR type 2, cytosol and microsomes were assayed with DHEA as the steroid substrate and either NAD^+/$NADP^+$ or NADH/NADPH as cofactor. Results from earlier studies of human lung fibroblast microsomes predict that if 17HSD/KSR type 2 is present, DHEA will be converted to 5-androstene-3β,17β-diol (5-ene-Adiol) [36]. As shown in Fig. 3, the predominant activity was a microsomal reductase leading to the conversion of DHEA to 5-ene-Adiol. NADH was the preferred cofactor. 3β-HSD/5-ene isomerase activity catalyzing the formation of A from DHEA was at the limit of detection.

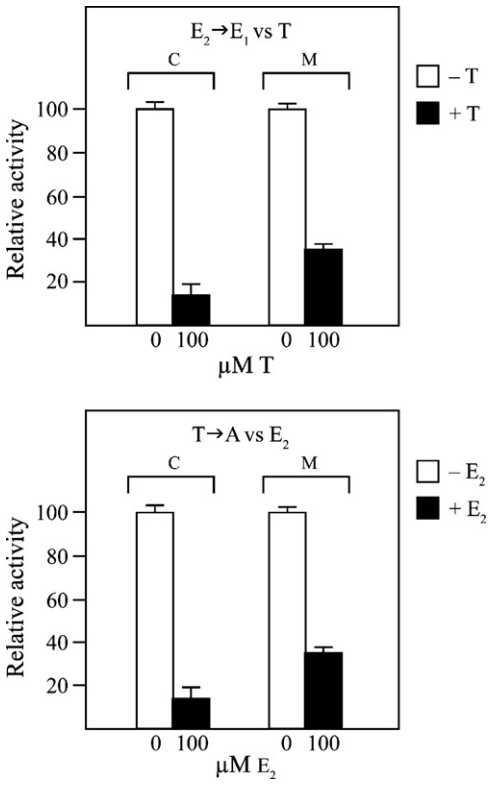

Fig. 2. Inhibition of 17HSD/KSR activity in OV-90 cell fractions. Inhibition of 17HSD/KSR activities with E_2 and T by T and E_2, respectively, was assayed in cytosolic (C) and microsomal (M) OV-90 cell fractions. Assays were run at pH 9.0 with 0.5 mM NAD^+, 1.0 μM substrate and with or without 100 μM inhibitor. The values are the mean ± SD of duplicate assays.

With 5α-DHT as substrate, there was a predominant NAD^+-dependent, microsomal dehydrogenase activity catalyzing the formation of 5α-androstane-3,17-dione (Adione). There was also a microsomal, NADH-dependent and cytosolic, NADPH-dependent 3α-HSD/KSR activity catalyzing the conversion of 5α-DHT to 5α-androstane-3α,17β-diol (3α-Adiol) (Fig. 4).

As shown in Fig. 5A, dehydrogenase activity with T in monolayer cultures of OV-90 cells is 12-fold greater than reductase activity with A, consistent with a predominant 17HSD/17KSR type 2 activity. A marked inhibition by E_2 and a lack of inhibition by A (Fig. 5B)

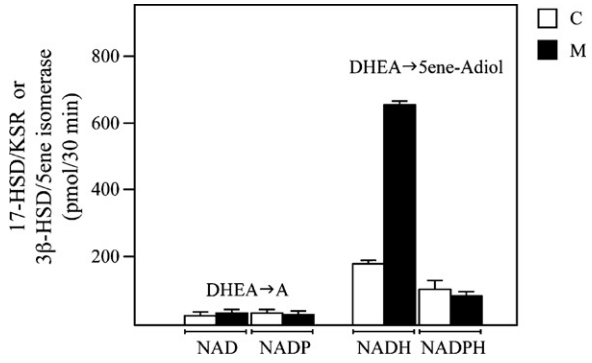

Fig. 3. DHEA metabolism in OV-90 cell fractions. DHEA metabolism was studied in cytosol (C) and microsomes (M) from approximately 3×10^6 OV-90 cells. Assays contained 0.5 mM cofactor and 1.0 μM DHEA in 0.04 M Hepes (pH 7.2). The values are the mean ± SD of duplicate assays.

Table 2
Effect of serum depletion on 17HSD/KSR activity of OV-90 cells.

Serum level	E_2 to E_1 (pmol/mg protein/30 min)	T to A (pmol/mg protein/30 min)
10%	3754 ± 397	1529 ± 57
10%/0.3%/0%	3937 ± 14	1493 ± 20

Control cultures were maintained in 10% serum for 7–10 days, until confluent. Test cultures were grown to confluence initially in 10% serum and then transferred to 0.3% serum for 7 days and to 0% serum for 24 h prior to sonication and assay. Assay conditions are given in the legend to Table 1. The values are for single cultures assayed in duplicate.

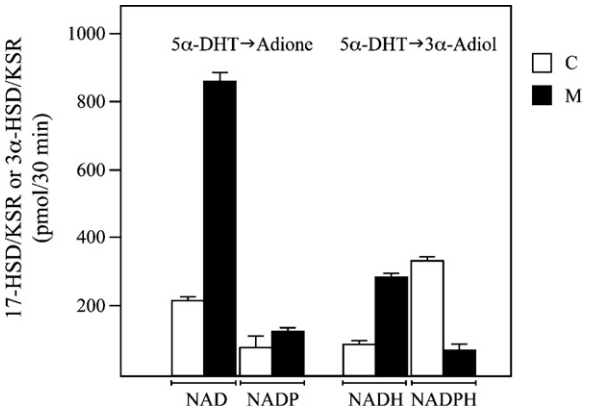

Fig. 4. 5α-DHT metabolism in OV-90 cell fractions. 5α-DHT metabolism was assayed in cytosol (C) and microsomes (M) from approximately 3.9×10^6 OV-90 cells. Assay conditions were the same as those given in the legend to Fig. 3, with 5α-DHT at 1.0 μM. The values are the mean ± SD of duplicate assays.

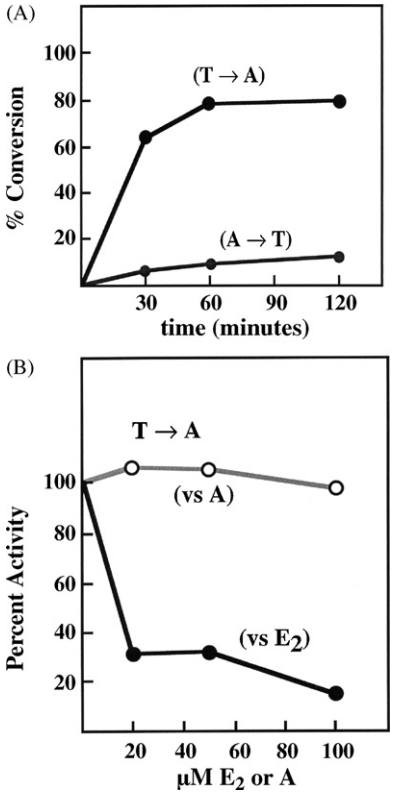

Fig. 5. Characterization of 17HSD/KSR type 2 activity in OV-90 intact cells. (A) Time course of T and A metabolisms by monolayers of OV-90 cells in 60-mm culture dishes. T and A were at 1.0 μM in DMEM/F-12 medium. (B) Effects of E_2 and A on T metabolism by monolayers of OV-90 cells. T was at 1.0 μM and cultures were incubated for 30 min.

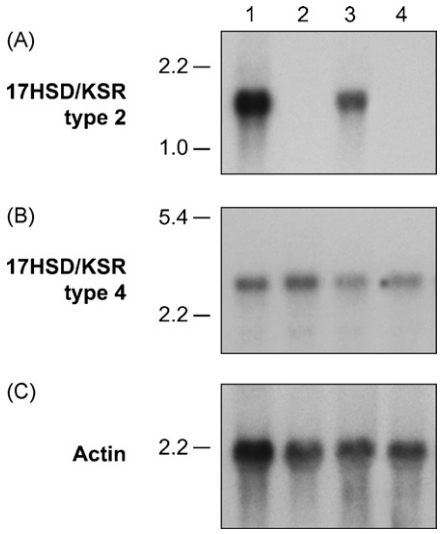

Fig. 6. Northern blot analysis of 17HSD/KSR type 2 and type 4 mRNA levels in OV-90 and TOV-21G cells. Fifteen μg of total RNA isolated from OV-90 (lane 1), TOV-21G (lane 2), A431 (lane 3), and A549 (lane 4) cells were hybridized with 17HSD/KSD type 2 (A), 17HSD/KSR type 4 (B), and γ-actin (C) specific probes. Position of the molecular weight standards (5.4, 2.2, and/or 1.0 kb) is shown. RNA from A431 and A549 cells were used as positive and negative controls, respectively. Panels A, B and C were from a 72-h, a 17-h, and a 1-h exposure, respectively.

are also consistent with the properties of 17HSD/17KSR type 2 in intact cells [37].

On the basis of data in the literature showing a marked effect of serum depletion on 17HSD/17KSR type 2 levels in human endometrial epithelial cell lines HEC-1-A and RL 95-2 [38], we tested whether 17HSD/17KSR type 2 levels are affected by serum depletion in OV-90 cells. As shown in Table 2, stepwise depletion of serum from the culture medium had no effect on dehydrogenase activity with either E_2 or T. In addition, exposure to insulin, 8-Br-cAMP or phorbol ester for 48 h in medium supplemented to 10% with serum had no effect on activity (data not shown).

3.2. Messenger RNA levels

17HSD/KSR type 2 mRNA was detected at high level in OV-90 but was not observed from TOV-21G cells by Northern blot analysis (Fig. 6). A431 cells which contain a high level of 17HSD/KSR type 2

Table 3
Comparison of expression levels between 17HSD/KSR type 1, type 2, and type 5 genes.

	17HSD/KSR (no. mRNA molecules/100 ng total RNA input)		
	Type 1	Type 2	Type 5
OV-90	2×10^2	4×10^3	1×10^4
TOV-21G	$<1 \times 10^1$	1×10^1	5×10^3

These values were obtained from QPCR using a specific standard curve for each mRNA. They are not normalized for the efficiency of reverse transcriptase reactions with values from housekeeping genes but, for each cell type, the same first strand cDNA preparation was used for the three genes.

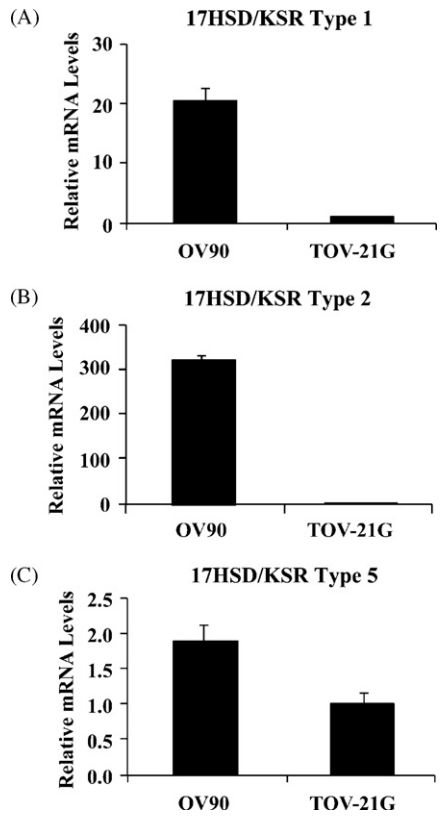

Fig. 7. Relative expression levels of 17HSD/KSR type 1, type 2, and type 5 in OV-90 and TOV-21G. QPCR analysis was performed in duplicate (±SD). All the values are normalized using four housekeeping genes. Values obtained for TOV-21G were fixed at 1-fold.

[37] and A549 cells which lack the type 2 isoform [24] were used as controls. The level of 17HSD/KSR type 2 mRNA was 3.1-fold higher in OV-90 compared to A431 cells after normalization with γ-actin mRNA levels. In contrast, a high level of type 4 mRNA was detected in both OV-90 and TOV-21G cells (Fig. 6).

Expression levels of 17HSD/KSR type 1, type 2, and type 5 in OV-90 and TOV-21G were studied by QPCR. 17HSD/KSR type 5 was expressed in both cell types with a difference of 1.9-fold in mRNA levels in favor of OV-90 (Fig. 7). In contrast, only trace amounts of 17HSD/KSR type 1 and type 2 mRNAs were detected in TOV-21G, while levels of these messengers in OV-90 exceeded these of

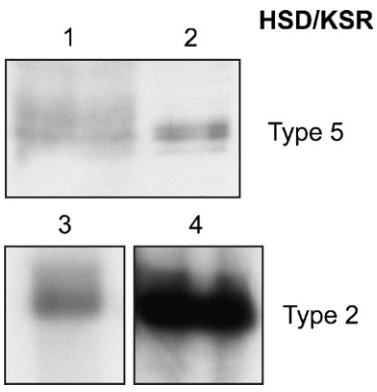

Fig. 8. Western blot analysis of 17HSD/KSR type 2 and type 5 in OV-90 cells. Lanes 2 and 4: total protein extract prepared using non-confluent OV-90 cells and corresponding to 50,000 cells per lane. HK-293 cells transfected with plasmids encoding for HSD/KSR type 2 (lane 3) and type 5 (lane 1) were used as positive controls. Anti-HSD/KSR type 2 (lanes 3 and 4) or type 5 (lanes 1 and 2) were used. Lanes 1, 2, and 4 were revealed together by chemiluminescence.

TOV-21G by more than 20- and 300-fold, respectively (Fig. 7). In order to compare expression levels between genes, the amounts of mRNA molecules per 100 ng total RNA input were calculated from QPCR data (Table 3). In OV-90, levels of 17HSD/KSR type 5 mRNA exceeded those of type 2 and type 1 by 2.5- and 50-fold, respectively, while the number of 17HSD/KSR type 2 mRNA molecules was 20-fold higher than that of the type 1.

3.3. Western blot analysis

HSD/KSR type 2 and type 5 were analyzed by Western blot in OV-90 cells. Both proteins were present (Fig. 8). The signal obtained for the type 2 was more intense than that of the type 5, but the relative affinity/avidity of the two antibodies is unknown.

4. Discussion

The spontaneously immortalized epithelial cell lines characterized in this study are of particular interest because they were derived from patients naïve to radiation and chemotherapy. Since their establishment in 1994 [22], they have been the subject of numerous studies directed toward gene expression analysis [39] and proteome profiling [40]. To our knowledge, ours is the first study to focus on 17HSD/KSR activity patterns and mRNA expression in these cell lines.

Previous results from our laboratories demonstrated the presence of 17HSD/KSR type 2 mRNA and activity in benign and malignant ovarian tumor tissue [19,20]. In agreement with an earlier report [41], activity levels varied widely between samples, and it was not possible to determine whether activity was localized to tumor cells. 17HSD/KSR type 1 immunoreactivity has been reported in non-luteinized stromal cells of mucinous ovarian tumors [42].

In the current study, one of the cell lines, OV-90, which was established from a malignant ovarian ascites from a patient with an adenocarcinoma, expressed a high level of constitutive 17HSD/KSR type 2 mRNA that correlated with a high level of type 2-like activity on the basis of its kinetic characteristics. This confirms that 17HSD/KSR type 2 can occur at high levels in epithelial cancer cells and also confirms a relationship between a high level of mRNA expression and enzymatic activity in this cell line. The relationship between mRNA expression levels and 17β-HSD type 2 activity levels in tumor cells has been of long-standing interest [43,44]. Interestingly, the situation does not seem to be the same for 17HSD/KSR type 5 because the type 5 mRNA level was 2.5-fold higher than that of the type 2 in OV-90 (Table 3) while the type 2-like enzymatic activity is clearly predominant compared to that of the type 5 (Fig. 5). Results from Western blot analysis (Fig. 8) confirm that the HSD/KSR type 2 protein was abundant and that the HSD/KSR type 5 protein was present in OV-90 cells.

Because there is a growing body of evidence of a role for 17HSD/KSR type 2 in the regulation of estrogen, androgen and progesterone receptor action in prostate [45], breast [46], colon [47] and ovarian cancer cells [20], cell lines expressing a high level of this isoform would appear to be of particular value as systems for evaluating the effects of enzyme-specific inhibitors on tumor cell survival. The OV-90 cell line should be used for that purpose. In addition because tumor recurrence and long-term survival correlate in some instances with the level of type 2 mRNA expression [43], clarification of the signaling pathways and transcription factors that regulate expression may be a fruitful approach to designing gene-specific therapies.

It is notable in our study that decreasing the serum level in the culture medium had no effect on the level of 17HSD/KSR activity in

OV-90 cells. Husen et al. [38] have shown that 17HSD/KSR type 2 mRNA expression levels in two human endometrial epithelial cell lines, HEC-1-A and RL 95-2, were markedly reduced concurrent with reductions in serum levels in the culture medium while type 4 mRNA expression was increased. More recent findings demonstrate that paracrine factors of stromal origin can affect 17HSD/KSR type 2 gene expression by influencing levels of the transcription factor specific protein-1 in human endometrial epithelial cells [48,49]. Fiorelli et al. [47] reported that replacement of fetal calf serum with charcoal-dextran-treated fetal calf serum had a variety of effects on 17HSD/KSR activities in four human colorectal cancer cell lines [47]. Although the basis for the stable, high level of type 2 mRNA expression in OV-90 cells remains to be clarified, it suggests the OV-90 cell line may be particularly useful for comparative studies.

17HSD/KSR type 2 functions as a dehydrogenase in intact cells under non-equilibrium conditions [37]. At equilibrium in intact cells, rates of dehydrogenase and reductase activity are equal [50]. Acting as a dehydrogenase, 17HSD/KSR type 2 would tend to be anti-estrogenic and anti-androgenic in that it would metabolize E_2 and T to the less hormonally active E_1 and A, respectively. In contrast, because of its 20α-HSD activity with C_{21}-steroids, conversion of 20α-dihydroprogesterone to progesterone would tend to stimulate progesterone action. These properties could have important consequences in ovarian epithelial tumors. Although there is a large body of information with regard to levels and distribution of estrogen, androgen and progesterone receptors in ovarian epithelial tumors, their role in the carcinogenesis of ovarian cancer remains controversial. A recent immunohistochemical study concluded that overexpression of progesterone receptor was associated with better survival [51]. Low levels of 17HSD/KSR type 2 mRNA expression have been associated with a favorable prognosis in female patients with distal colorectal cancer [52] whereas retained expression of 17HSD/KSR type 2 mRNA was associated with a decreased risk of late recurrence in estrogen receptor positive breast cancer patients [43].

Although 17HSD/KSR type 2 is the predominant activity in OV-90 cells, 10–30% of the activity did not show a type 2 inhibition pattern. The remaining 17HSD/KSR activity may originate from the type 5 enzyme since 17HSD/KSR type 5 mRNA and protein were observed in OV-90 cells and that 17HSD/KSR activity in cytosol was observed with E_2, T (Fig. 1), and DHEA (Fig. 3) as substrates, which is a characteristic of the type 5 activity. Expression of 17HSD/KSR type 1 and type 7 mRNAs has been reported for ovarian surface epithelial cells [15]. While 17HSD/KSR type 7 could also account for a part of the remaining activity, 17HSD/KSR type 1 does not seem to contribute significantly to this activity since the E_2/T activity ratio is not characteristic of 17HSD/KSR type 1 activity. We also observed a significant level of microsomal and cytosolic 3α-HSD/KSR activity. The cytosolic activity could reflect the presence of 17HSD/KSR type 5, which has 3α-HSD/KSR activity [8]. A microsomal NADH-dependent activity has also been described [53].

Further analyses are required for better understanding the endocrinology of ovarian cancer cell lines. They should include the use of specific enzyme inhibitors such as the 17HSD/KSR type 1 inhibitor recently reported [54].

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